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(54) Title: DNA SEQUENCES ENCODING SEMAPHORIN-H AND DIAGNOSIS OF METASTATIC CANCER

(57) Abstract

The present invention relates to DNA molecules encoding novel members of the Semaphorin family, referred to as Semaphorin-H and Semaphorin-H-v. The invention further relates to polypeptides encoded by the DNA, to antibodies that bind to the polypeptides and to compositions and methods comprising the DNA, polypeptides and antibodies. The present invention provides methods for detecting metastatic cancer and determining the metastatic potential of cells. The invention contemplates the development of cells line with varying metastatic potential for use a models for studying, diagnosing and treating metastatic disease.

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DNA SEQUENCES ENCODING SEMA-H AND
DIAGNOSIS OF METASTATIC CANCER

TECHNICAL FIELD OF THE INVENTION

The invention relates to DNA molecules

- 5 comprising sequences encoding a novel member of the Semaphorin family, Semaphorin-H. The invention further relates to the polypeptides encoded by those DNA sequences and antibodies that bind to those polypeptides. The invention also relates to
- 10 recombinant DNA molecules comprising these DNA sequences, as well as hosts transformed with such recombinant DNA molecules. The invention further relates to the use of the DNA molecules, polypeptides and antibodies of this invention in the study,
- 15 diagnosis and treatment of metastasis.

The present invention further provides methods for the diagnosis of metastatic cancer by detection of the sema-H mRNA or the sema-H protein encoded by the sema-H gene. The present invention

- 20 contemplates the use of recombinant sema-H DNA and antibodies directed against the sema-H protein to determine the metastatic potential of several types of tumor cells, including, for example, thyroid, epithelial, lung, liver, kidney, breast, lymphoid,
- 25 hematopoietic, pancreatic, endometrial, ovarian, cervical, skin, colon and similar tumor cells.

The present invention also provides mammalian cell lines and tumors with high and low metastatic potential developed through use of the disclosed

- 30 invention. Such cell lines to serve as useful model systems for in vitro and in vivo anti-metastasis drug screening.

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systems for in vitro and in vivo anti-metastasis drug screening.

BACKGROUND OF THE INVENTION

Malignant cancer tumors shed cells which 5 migrate to new tissues and create secondary tumors; a benign tumor does not generate secondary tumors. The process of generating secondary tumors is called metastasis and is a complex process in which tumor cells colonize sites distant from the primary tumor. 10 Tumor metastasis remains the major cause of morbidity and death for patients with cancer. One of the greatest challenges in cancer research is to understand the basis of metastasis, i.e., what controls the spread of tumor cells through the blood and lymphatic systems 15 and what allows tumor cells to populate and flourish in new locations.

The metastatic process appears to be sequential and selective, and is controlled by a series of steps since metastatic tumor cells: (a) are mobile 20 and can disseminate from the original tumor; (b) are capable of invading the cellular matrix and penetrating through blood vessels; (c) possess immunological markers, which allow them to survive passage through the blood stream, where they must avoid the 25 immunologically active cytotoxic "T" lymphocytes; and (d) have the ability to find a favorable location to transplant themselves and successfully survive and grow.

Understanding the underlying molecular 30 mechanisms in metastasis is the key to understanding cancer biology and its therapy. In clinical lesions,

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malignant tumors contain a heterogeneous population of cells, exhibiting a variety of biological characteristics, e.g., differential growth rates, cell surface structures, invasive capacities and sensitivity

5 to various cytotoxic drugs. Researchers can take advantage of tumor heterogeneity factors, by identifying specific cell produced markers, which are unique for metastasis, to develop therapeutic regimens which do not rely only on surgical resection alone.

10 At this time it is not known whether the metastatic phenotype is under the regulation of a single gene or multiple genes, which genes may be independent or interrelated. However, a number of genes have become correlated with the formation and

15 metastasis of tumors. For example, several normal cellular genes become oncogenes by incorporation into a retroviral genome. Due to the juxtaposition of new promoter elements, such incorporation frequently allows potential oncogene to be expressed in inappropriate

20 tissues or at higher levels than it normally would be expressed. It appears from work with tumorigenic retroviruses as well as other systems, that misexpression of many cellular proteins, particularly those involved in the regulation of the cell cycle,

25 cell mobility, or cell-cell interaction may lead to a cancerous phenotype. It is, therefore, important to identify genes involved in the biological pathways of metastasis as this identification is critical to the development of preventative, diagnostic and treatment

30 measures.

The Semaphorin/Collapsin family of molecules was discovered recently and is characterized by unique and highly conserved motifs within a 500 amino acid semaphorin domain. Proteins of the family all contain

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a signal sequence and are either transmembrane or secreted. Grasshopper SemaI, Drosophila SemaII, mouse SemaD, chicken collapsinI and mouse SemaIII exhibit inhibitory or repulsive functions in a neuronal 5 context. However, the wide expression of some semaphorin members indicates alternative functions for the proteins. SemaIII knockout mice, for example, show abnormal bone structure, in addition to neural abnormalities.

10 Data also implicate semaphorins in the pathogenesis of disease. A semaphorin domain is encoded by variola, vaccinia and acelaphine herpes virus 1. The sema IV and semaV genes are located in the 3p21.3 chromosomal region in humans, a region that 15 is deleted in many small cell lung cancer tumors.

16 The present invention discloses the mouse sema-H gene and its usefulness in the diagnosis of metastatic cancer by use of either antibodies directed against the sema-H protein or sema-H nucleic acid 20 probes directed against sema-H mRNA.

SUMMARY OF THE INVENTION

25 The present invention provides semaH polypeptides, and fragments and derivatives thereof as well as nucleic acids encoding them. Another aspect of the invention provides antibodies that specifically bind to semaH polypeptides of the invention. A further aspect of the invention provides antisense oligonucleotides derived from the nucleic acid 30 sequences of the invention. The present invention also provides methods and compositions for detecting and preventing metastasis utilizing the polypeptides,

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nucleic acids, antisense oligonucleotides and antibodies of the invention.

Additionally, the present invention provides recombinant DNA molecules comprising the DNA sequences 5 of the invention, host cells transformed with the recombinant DNA molecules and methods for producing the polypeptides encoded by those DNA sequences utilizing the transformed host cells.

Therefore, one aspect of the present 10 invention provides an isolated, recombinant nucleic acid encoding a human Semaphorin-H gene or a fragment thereof, and replicable DNA sequences encoding a Semaphorin-H polypeptide which express high or low levels of the Semaphorin-H polypeptide. Isolated 15 antisense Semaphorin-H nucleic acids and expression vectors are also contemplated by the present invention. Human Semaphorin-H nucleic acids are preferred. The DNA sequences of this invention may be used in both therapeutic and diagnostic applications. The DNA 20 sequences encoding inactive, mutant or truncated forms of Semaphorin-H are useful in gene therapy to prevent metastasis of cancer cells by competing with the native, active form of the protein in the cell.

A further aspect of this invention provides 25 isolated transformed host cells, such as prokaryotic microorganisms, yeast, insect cells and eukaryotic cells, containing Semaphorin-H nucleic acids and replicable vectors containing DNA sequences encoding the Semaphorin-H polypeptide.

30 A still further aspect of this invention provides isolated mammalian Semaphorin-H polypeptides and pharmaceutical compositions comprising them. Human Semaphorin-H polypeptides are preferred.

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The DNA sequences encoding Semaphorin-H are suitable for use in creating transformed cell lines with high metastatic potential, especially in cancer cell lines, for example, for use in research.

5 The DNA sequences of this invention may be used as diagnostic tools to detect and quantify Semaphorin-H mRNA levels in various cells. This method may be used to determine the metastatic potential of cancer cells, a high Semaphorin-H mRNA level suggesting 10 that the cell has high metastatic potential.

The Semaphorin-H DNA sequences of this invention may also be used to inhibit the expression of Semaphorin-H polypeptides in a cell through the use of anti-sense technology. Single stranded, anti-sense DNA 15 can be introduced into cells where it can hybridize to and inhibit the translation of Semaphorin-H mRNA. Such methods can be used to prevent a cancer cell from metastasizing.

The invention also provides monoclonal and 20 polyclonal antibodies directed against a Semaphorin-H polypeptide or any peptide, fragment or derivative of the Semaphorin-H protein. These antibodies may be used to assay for Semaphorin-H levels in a patient or cell. In addition, Semaphorin-H antibodies are useful in 25 inhibiting Semaphorin-H activity, and therefore, metastasis. One aspect of the present invention is directed to a method for diagnosing metastatic cancer by contacting serum from an individual to be tested for such cancer with an antibody reactive with a mammalian 30 Semaphorin-H protein or an antigenic fragment thereof, for a time and under conditions sufficient to form an antigen-antibody complex, and detecting the antigen-antibody complex.

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A further aspect of this invention is directed towards treatment of cancer by administering reagents, such as for example, anti-Semaphorin-H antibodies capable of binding the Semaphorin-H protein 5 and antisense Semaphorin-H nucleic acids capable of binding Semaphorin-H sense mRNA.

Yet another aspect of the present invention provides an animal model system of the metastatic process, including several eukaryotic cell lines and 10 tumors expressing different levels of Semaphorin-H, which can be derived, for example, from mouse and rat carcinomas. These cell lines and tumors may be re-introduced, for example, into mice or rats to produce primary tumors which metastasize to the lung, liver and 15 kidneys with a characteristic frequency. Therefore, the present invention also provides a well controlled animal model system for testing pharmaceutical compositions suspected to have therapeutic utility for the treatment of metastatic cancer.

20

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Identification of M-semaH transcripts in metastatic cell lines

(A) Differential display showing the amplification of a 525 bp fragment from the two metastatic cell lines 25 66c14 and 4T1 (arrow).

(B) Confirmation of the differential expression by Northern hybridization of the 525 bp fragment to RNA from tumor cell lines. 10 µg total RNA was loaded in each lane.

30 (C) Northern hybridization of the 525 bp fragment to various mouse tumor cell lines. Note the degree of correlation with metastatic potential in figures B and

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C as well as the presence of 4.0 kb, 4.5 kb and -7.0 kb transcripts.

^aVMR-Ly was found to be metastatic to the lymph nodes but not the lungs. The presence of transcripts was 5 confirmed by prolonged exposition (data not shown).

^bNot determined.

S.C.= spontaneous metastasis assay, injection in the subcutis; i.v. = experimental metastasis assay, injection intravenously; +=metastatic; -- 10 nonmetastatic.

Figure 2: The difference between the 4.5 kb and 4.0 transcripts

(A) Diagram depicting the 4466 bp and the 3989 bp cDNA fragments obtained by library screening. Binding sites 15 for different probes (A, B, C, D) are indicated above. Probe A corresponds to the 525 bp fragments obtained from the display in Figure 1A. Arrows indicate the position of primers in the 5' RACE. The interleaved lines indicate the additional sequences retrieved from 20 the 5' RACE.

(B) Northern hybridization of probe B to RNA from tumor cell lines 4T1 (lane 1), 66c14 (lane 2), 4T07 (lane 3).

(C) Northern hybridization using probe C to the same 25 RNA as in (B). Note that the region missing in the 3675 bp cDNA fragment is also absent in the 4.0 kb *M-sema H-v* transcript.

(D) Northern hybridization with probe D to the same RNA as in (B). The presence of the 4.0 kb *M-sema H-v* 30 transcript shows that this transcript shares the 5'-end with the 4.5 kb transcript.

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Figure 3: Expression of *M-semaH* transcripts in mouse adult tissues and whole embryos.

(A) Upper: Northern hybridization to total RNA from tissues of A/Sn mice using probe A (see Figure 2A).
5 Note the absence of expression in normal mammary tissue. Lower: control using a GAPDH probe.

(B) Upper: Northern hybridization to total RNA of whole A/Sn embryos between 10.5-18.5 dpc of mouse development as well as from the head and back of a 15.5 dpc embryo
10 and the brain of a newborn mouse. Same probe as in (A). Note the expression before day 13.5. Lower: control using 32 P-labeled poly(U). (A and B) Both filters were exposed for one week. The position of the 4.0 kb transcript in the 66c14 lanes is recognizable
15 from a 24 h exposure placed next to the filter and marked with an asterisk.

Abbreviations: B, back; H, head; NB, newborn; dpc, day(s) post coitum.

Figure 4: Conserved motifs in the predicted M-SemaH sequence

(A) Alignment of the Sema domains of M-SemaH, C-Coll 5, H-Sema III, D-SemaII, and G-SemaI. The alignment was made using the Pileup program (Genetics Computer Group). Conserved cysteine residues are marked with asterisks. Potential N-glycosylation sites in M-SemaH are marked with number symbols. Conserved amino acids are boxed.
25 (B) Diagram depicting the organization of the proteins aligned in (A). The percentages refer to the degree of identity between the entire amino-acid sequence of M-SemaH and the proteins shown as calculated using the
30

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Gap program (Genetics Computer Group). A small black bar in the N-terminal means that a signal peptide is present. A plus in the C-terminal means that 18-20 positively charged residues are present within the last 5 50 aa. Sema, Semaphorin; Ig, immunoglobulin-like domain; TM, transmembrane domain. M, mouse; C, chick; Coll, Collapsin; H, human; D, *drosophila*; G, Grasshopper.

10 **Figure 5: *In situ* hybridization analysis of *M-semaH* expression during mouse development.**

Darkfield illuminations of hybridizations with an *M-semaH/H-v* antisense probe (A, C, E, G) Brightfield illuminations of sections succeeding A, C, E, G stained with haematoxylin-eosin (he) (B, D, F, H). (A-D) 15 Transversal section through the body of a 12.5 dpc embryo.

(A) The *M-semaH* transcripts are expressed in the ventral horns (arrow heads) of the neural tube and in sclerotomal cells underlying the spinal ganglia.

20 (B) A section following (A) stained with He.

(C) The section succeeding (A) showing sclerotomal signals but no signals in the ventral horns.

(D) He-staining of section succeeding (C).

(E) Parasagittal section of the lower body of a 14 dpc 25 embryo. The *M-semaH* signals are visible in the developing intervertebral discs.

(F) He-staining of the section succeeding E. Arrow heads in (E) and (F) indicate the positions of the intervertebral discs.

30 (G) Sagittal section through the lung of a 16 dpc embryo. The *M-semaH* transcripts are detectable in the epithelium of the bronchi.

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(H) He-staining of a section succeeding G.

Bv, blood vessel; br, bronchus, id, primordium of the intervertebral discs; nt, neural tube; sg, spinal ganglia; vb, primordium of the vertebral body.

5

DETAILED DESCRIPTION OF THE INVENTION

The following definitions are used throughout the specification and the claims.

"Sema-H" refers to nucleotide sequences, 10 either DNA or RNA, single-stranded or double-stranded that encode a Sema-H polypeptide or derivative thereof. When used to define a double-stranded nucleotide sequence, the term also refers to the anti-sense nucleotide sequence.

15 "Sema-H polypeptide" refers to a polypeptide which is at least 80%, and more preferably, at least 90% identical in amino acids 29-303 of SEQ ID NO:3 or SEQ ID NO:4.

Additional terms are defined where necessary 20 throughout the application.

According to one embodiment, the invention provides isolated DNA molecules comprising the DNA sequences of SEQ ID NO:1 and SEQ ID NO:2 or fragments thereof; DNA sequences that hybridize under stringent 25 conditions to SEQ ID NO:1 and SEQ ID NO:2 or fragments thereof; DNA sequences which encode a polypeptide having the same amino acid sequences encoded by SEQ ID NO:3 or SEQ ID NO:4 or a fragment thereof; and sequences which hybridize thereto under stringent 30 conditions.

As used herein, the term "hybridize to under stringent conditions" refers to the ability of a

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denatured DNA sequence to hydrogen bond to another denatured DNA sequence through complementary base pairs under conditions which allow sequences having at least 80% similarity to form such hybrids. Such conditions 5 are well known in the art and are exemplified by salt and temperature conditions substantially equivalent to 5X SSC and 65° for both hybridization and wash.

Besides the full-length cDNA sequences set forth herein, it will be readily apparent to those of 10 skill in the art that any other DNA sequence which, as a result of degeneracy in the genetic code, encodes the same amino acid sequence as SEQ ID NO:3 or SEQ ID NO:4 is part of applicant's invention. While those specific sequences are not set forth herein due to space 15 considerations, it should be understood that one of ordinary skill in the art could ascertain all of such DNA sequences merely by reference to the genetic code and without the exercise of inventive skill.

In addition to genetically redundant DNA 20 sequences, the invention also includes DNA sequences which encode other amino acid sequences which are at least 80%, and preferably at least 90% similar to SEQ ID NO:3 or SEQ ID NO:4. Because this aspect of the invention does not require the isolated DNA sequence to 25 encode an active Sema-H, any nucleotides of SEQ ID NO:1 and SEQ ID NO:2 may be modified to produce a DNA sequence of this invention. The identification and isolation of additional sequences may be achieved by standard DNA library screening techniques 30 (hybridization, PCR) using SEQ ID NO:1 and SEQ ID NO:2 or portions thereof as a probe. Such homologous sequences may be found in any mammalian tissue cDNA library, as well as in insect cDNA libraries, yeast and

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other fungi cDNA libraries and prokaryotic cDNA libraries.

According to an alternate embodiment, the invention provides for isolated DNA sequences which 5 encode Sema-H polypeptides; DNA sequences which hybridize to either of the former DNA sequences; and DNA sequences which code for a polypeptide having the same amino acid sequence as any of the previous DNA sequences.

10 Additional DNA sequences which encode Sema-H polypeptides may be identified by standard DNA library screening techniques using nucleotides of SEQ ID NO:1 and SEQ ID NO:2 or portions thereof as a probe. Even more preferred are homologous DNA sequences which 15 contain nucleotides encoding only conservative amino acid substitutions for some or all of the other amino acids of SEQ ID NO:3 or SEQ ID NO:4. The translation products of any of these DNA sequences may then be used in assays or methods as described below.

20 As set forth above, DNA sequences according to this aspect of the invention may be identified and isolated using methods well known in the art, for example, through standard cDNA library screening. It will be appreciated by one of skill in the art that 25 screening techniques such as those described in the art may be used to identify homologous genes in other species, and these DNA and amino acid sequences are also included in the present invention.

According to another embodiment of this 30 invention, peptide fragments of Sema-H can be generated from the full length sequence using chemical or recombinant DNA techniques. Alternatively, or in combination, synthetic fragments of DNA or protein which represent portions of the Sema-H sequences

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identified above may be generated. In a preferred embodiment, these fragments represent unique portions of Sema-H in that they are not found in any previously known protein. A unique portion would preferably have

5 an amino acid sequence length at least long enough to define a novel peptide. Depending on the particular amino acid sequence, this unique portion would preferably consist of about 5 to about 25 amino acids, or most preferably about 5 to about 10 amino acids.

10 These unique portions can be identified by comparing the amino acid sequence of Sema-H with known data base sequences.

As would be known to those skilled in the art, these unique portions, or any of the sequences named herein, may be free or coupled to other atoms or molecules, or they may be contiguous with a larger polypeptide derived from any source. They may be modified or joined to other compounds using any technique known to those skilled in the art, including but not limited to, physical, chemical or molecular techniques. These modifications may affect properties of the Sema-H or Sema-H derived polypeptides in a manner predictable to those with skill in the art. These properties may include solubility, stability, binding specificity, affinity, toxicity, localization, detectability, half life, targeting, bioavailability, antibody reactivity, protein folding, etc. and would be readily identifiable using appropriate assays.

According to another embodiment of this invention, any of the DNA sequences described above may be employed in a recombinant DNA molecule. The isolated DNA sequences of this invention may be inserted into any of the numerous commercially or otherwise publicly available cloning vectors. Useful

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expression vectors, for example, may consist of segments of chromosomal, nonchromosomal or synthetic DNA sequences. Suitable vectors include, but are not limited to, derivatives of SV40 and known bacterial 5 plasmids, e.g., E.coli plasmids col El, pCR1, pBR322, pMB9 and their derivatives, plasmids such as RP4; phage DNAs, e.g., the numerous derivatives of phage λ , e.g., NM989, and other phage DNA, e.g., M13 and Filamentous single stranded phage DNA; yeast plasmids such as the 10 2μ plasmid or derivatives thereof; vectors useful in eukaryotic cells, such as vectors useful in insect or mammalian cells; vectors derived from combinations of plasmids and phage DNAs, such as plasmids that have been modified to employ phage DNA or other expression 15 control sequences; and the like.

The choice of cloning vector will, of course, depend upon the cell type(s) to be transformed with the resulting recombinant DNA molecule, as well as the ultimate use of that recombinant DNA molecules (i.e., 20 DNA production, gene therapy, polypeptide expression). Preferred eukaryotic vectors are SV40-derived vectors. Preferred prokaryotic vectors are E. coli expression vectors. Preferred viral vectors are modified eukaryotic viral vectors, preferably attenuated 25 adenovirus vectors, that may be used in gene therapy, such as those described in PCT publications WO 94/26915 and WO 94/28938, the disclosures of which are herein incorporated by reference.

Techniques for inserting a DNA sequence of 30 this invention into a vector to produce a recombinant DNA molecule involve standard molecular biological techniques and are well known in the art (see, for example, J. Sambrook, et al., Molecular Cloning, A

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Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)).

According to a preferred embodiment, the recombinant DNA molecule of this invention will 5 additionally comprise an expression control sequence operatively linked to a DNA sequence of this invention. The term "expression control sequence" refers to a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. It includes 10 both 5' and 3' non-coding DNA sequences and optionally includes an ATG start codon.

The term "operatively linked" refers to the positioning of an expression control sequence with respect to a coding DNA sequence of interest such that 15 the expression control sequence controls and regulates the transcription and translation of that DNA sequence. The term "operatively linked" includes having an appropriate start signal (e.g., ATG) in front of the DNA sequence to be expressed and maintaining the 20 correct reading frame to permit expression of the DNA sequence under the control of the expression control sequence and production of the desired product encoded by the DNA sequence. If a gene or DNA sequence that one desires to insert into a recombinant DNA molecule 25 does not contain an appropriate start signal, such a start signal can be inserted in front of the gene.

The choice of expression control sequence depends upon the nature of the recombinant DNA molecule, the host that will be transformed by that 30 recombinant DNA molecule, and whether constitutive or inducible expression of a DNA sequence of this invention is desired. Such useful expression control sequences include, for example, the early and late promoters of SV40 or adenovirus, the lac system, the

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trp system, the TAC or TRC system, the major operator and promoter regions of phage λ , the control regions of fd coat protein, the promoter for 3-phosphoglycerate kinase or other glycolytic enzymes, the promoters of acid phosphatase (e.g., Pho5), the promoters of the yeast α -mating factors, and other sequences known to control the expression of genes of prokaryotic or eukaryotic cells or their viruses, and various combinations thereof.

10 Preferably the vector also encodes a selectable marker, for example, antibiotic resistance. Replicable expression vectors can be plasmids, bacteriophages, cosmids and viruses. Any expression vector comprising RNA is also contemplated.

15 Preferred vectors of the present invention are derived from eukaryotic sources. Expression vectors that function in tissue culture cells are especially useful, but yeast vectors are also contemplated. These vectors include yeast plasmids and 20 minichromosomes, retrovirus vectors, BPV (bovine papilloma virus) vectors, baculovirus vectors, SV40 based vectors and other viral vectors. SV40-based vectors and retrovirus vectors (e.g., murine leukemia viral vectors) are preferred. Tissue culture cells 25 that are used with eukaryotic replicable expression vectors include Sf21 cells, CV-1 cells, COS-1 cells, NIH3T3 cells, mouse L cells, HeLa cells and such other cultured cell lines known to one skilled in the art.

30 A baculovirus expression system can be used to produce large amounts of Sema-H polypeptides in cultured insect cells. The post-translational processing of polypeptides produced in such insect cells is similar to that of mammalian cells. Production of polypeptides in insects is therefore

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advantageous, particularly when one seeks to mimic the exact function or antigenic properties of the natural polypeptide.

Methods for producing polypeptides in the 5 baculovirus expression system are known to the skilled artisan. See for example Miller 1988 Ann. Rev. Microbiol. 42: 177. In general, a modified Autographa californica nuclear polyhedrosis virus propagated in Sf21 cells is used for polypeptide expression. This 10 modified virus is produced by cotransfection of a small transfer vector, encoding a Sema-H polypeptide, with a viral expression vector which has been linearized within an essential gene. Once inside the cell, the linearized expression vector can undergo recombination 15 with the transfer vector or simply recircularize. However, only recombination gives rise to viable viruses because the function of the essential gene is lost by recircularization. Recombinant expression viruses are detected by formation of plaques.

20 The present invention also contemplates prokaryotic vectors that may be suitable for expression of the mammalian Sema-H gene, including bacterial and bacteriophage vectors that can transform such hosts as *E. coli*, *B. subtilis*, *Streptomyces* sps. and other 25 microorganisms. Many of these vectors are based on pBR322 including Bluescript™ (commercially available from Stratagene) and are well known in the art. Bacteriophage vectors that are used in the invention include lambda and M13.

30 Sequence elements capable of effecting expression of the Sema-H gene include promoters, enhancer elements, transcription termination signals and polyadenylation sites. Promoters are DNA sequence elements for controlling gene expression, in

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particular, they specify transcription initiation sites. Prokaryotic promoters that are useful include the lac promoter, the trp promoter, and P_L and P_n promoters of lambda and the T7 polymerase promoter.

5 Eukaryotic promoters are especially useful in the invention and include promoters of viral origin, such as the SV40 late promoter and the Moloney Leukemia virus LTR, Murine Sarcoma Virus (MSV) LTR, yeast promoters and any promoters or variations of promoters
10 designed to control gene expression, including genetically-engineered promoters. Control of gene expression includes the ability to regulate a gene both positively and negatively (i.e., turning gene expression on or off) to obtain the desired level of
15 expression.

The replicable expression vectors of the present invention can be made by ligating part or all of the Sema-H coding region in the sense or antisense orientation to the promoter and other sequence elements
20 being used to control gene expression. This juxtapositioning of promoter and other sequence elements with the Sema-H gene allows the production of large amounts of sense or antisense Sema-H mRNA. Large amounts of the Sema-H protein can also be produced
25 which are useful not only for anti-Sema-H antibody production but also for analysis of the function of Sema-H in metastatic cancer as well as for designing therapies for metastatic cancer. Analysis of the Sema-H protein, in this embodiment, includes the use of
30 recombinant protein for use in crystallographic examination. Crystallographic examination of Sema-H could be used to identify small molecule inhibitors of active sites on the protein.

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Therefore, one skilled in the art has available many choices of replicable expression vectors, compatible hosts and well-known methods for making and using the vectors. Recombinant DNA methods 5 are found in any of the standard laboratory manuals on genetic engineering.

The invention also provides host cells transformed by the recombinant DNA molecules of this invention. These hosts may include well known 10 eukaryotic and prokaryotic hosts, such as strains of E.coli, Pseudomonas, Bacillus, Streptomyces, fungi such as yeasts, and animal cells, such as WHO, RLL, B-W and L-M cells, African Green Monkey kidney cells (e.g., COS 1, COS 7, BSC1, BSC40, and BMT10), insect cells (e.g., 15 Sf9), and human cells and plant cells in tissue culture. Eukaryotic cells may harbor the recombinant DNA molecules of this invention as an extra chromosomal element or incorporate all or part of it into the host chromosome.

20 It will be understood that not all vectors, expression control sequences and hosts will function equally well to replicate and/or express the DNA sequences of this invention. Neither will all hosts function equally well with the same expression system. 25 However, one skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention. For example, in selecting a vector, 30 the host must be considered because the vector must function in it. The vector's copy number, the ability to control that copy number, and the expression of any other proteins encoded by the vector, such as

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antibiotic and other selective markers, will also be considered.

In selecting an expression control sequence, a variety of factors will normally be considered.

- 5 These include, for example, the relative strength of the system, its controllability, and its compatibility with the particular DNA sequence or gene to be expressed, particularly as regards potential secondary structures. Suitable unicellular hosts will be
- 10 selected by consideration of, e.g., their compatibility with the chosen vector, their ability to fold proteins correctly, and their fermentation requirements, as well as the toxicity to the host of the product encoded by the DNA sequences to be expressed, and the ease of
- 15 purification of the expression products.

Considering these and other factors a person skilled in the art will be able to construct a variety of vector/expression control sequence/host combinations that will replicate and/or express the DNA sequences of this invention on fermentation or in large scale animal culture.

Methods for transforming cells with recombinant DNA molecules are well known in the art (see, for example, J. Sambrook, et al., Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989)). Any of those methods may be employed to produce the transformed hosts of this invention. Identification of transformed hosts may be achieved by assaying for the presence of Sema-H DNA, Sema-H RNA or Sema-H polypeptide. Additionally, transformants may be identified by growth in selective media. For this assay, the gene necessary for growth in selective media is cotransfected into the cell either on the same or a

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different recombinant DNA molecule as the Sema-H DNA and is expressible in that cell. It will, of course, be obvious that the gene for selective growth should not be present in the untransformed cell.

5 The transformed hosts of this invention may be employed to produce either large quantities of Sema-H DNA sequences of this invention and/or the Sema-H polypeptide encoded thereby. In order to produce large quantities of Sema-H DNA, the host, preferably a
10 prokaryotic host, is grown in a medium and under conditions that promote DNA replication and cell division. Any complete media routinely used to grow bacteria is suitable for this purpose. Following growth, the transformed cells are separated from the
15 growth medium and plasmid DNA is then isolated from the cells by standard and well-known techniques. Sema-H DNA may then be excised from the plasmid through the use of restriction endonucleases.

When the transformed hosts of this embodiment
20 are employed, the preferred host is a mammalian cell. The transformed host should be grown in a medium that promotes expression of the Sema-H polypeptide-encoding DNA sequence present in that host. If expression of that DNA sequence is under the control of a
25 constitutive promoter, any standard growth medium is suitable. If the Sema-H DNA is under the control of an inducible promoter, the growth medium should be supplemented with a compound that induces expression or growth conditions should be altered so as to induce
30 expression (i.e., change in growth temperature). Following expression, the transformed cells are separated from the growth medium, lysed and the Sema-H polypeptide is purified by standard methods. If the cells secrete the Sema-H polypeptide, the protein may

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be harvested directly from the media without cell lysis.

In a further embodiment, the present invention further provides derivatives of the Sema-H polypeptide. As used herein, a "derivative" of a Sema-H polypeptide according to his invention is a Sema-H polypeptide in which one or more physical, chemical, or biological properties has been altered. Such modifications include, but are not limited to: amino acid substitutions, modifications, additions or deletions; alterations in the pattern of lipidation, glycosylation or phosphorylation; reactions of free amino, carboxyl, or hydroxyl side groups of the amino acid residues present in the polypeptide with other organic and non-organic molecules; and other modifications, any of which may result in changes in primary, secondary or tertiary structure.

In accordance with this invention, derivatives of the novel Sema-H polypeptides may be prepared by a variety of methods, including by *in vitro* manipulation of the DNA encoding the native polypeptides and subsequent expression of the modified DNA, by chemical synthesis of derivatized DNA sequences, or by chemical or biological manipulation of expressed amino acid sequences.

For example, derivatives may be produced by substitution of one or more amino acids with a different natural amino acid, an amino acid derivative or non-native amino acid, conservative substitution being preferred, e.g., 3-methyl histidine may be substituted for histidine, 4-hydroxyproline may be substituted for proline, 5-hydroxylysine may be substituted for lysine, and the like.

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Causing amino acid substitutions which are less conservative may also result in desired derivatives, e.g., by causing changes in charge, conformation and other biological properties. Such 5 substitutions would include for example, substitution of a hydrophilic residue for a hydrophobic residue, substitution of a cysteine or proline for another residue, substitution of a residue having a small side chain for a residue having a bulky side chain or 10 substitution of a residue having a net positive charge for a residue having a net negative charge. When the result of a given substitution cannot be predicted with certainty, the derivatives may be readily assayed according to the methods disclosed herein to determine 15 the presence or absence of the desired characteristics.

In a preferred embodiment of this invention, the Sema-H polypeptides disclosed herein are prepared as part of a larger fusion protein. For example, a Sema-H polypeptide of this invention may be fused at 20 its N-terminus or C-terminus to a different immunogenic Sema-H polypeptide, to a non-Sema-H polypeptide or to combinations thereof, to produce fusion proteins comprising the Sema-H polypeptide.

The Sema-H polypeptides may also be part of 25 larger multimeric molecules which may be produced recombinantly or may be synthesized chemically. Such multimers may also include the polypeptides fused or coupled to moieties other than amino acids, including lipids and carbohydrates.

30 It will be readily appreciated by one of ordinary skill in the art that the Sema-H polypeptides of this invention, as well as fusion proteins and multimeric proteins containing them, may be prepared by

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recombinant means, chemical means, or combinations thereof.

The molecules comprising the Sema-H polypeptides encoded by the DNA sequences of this invention, or derivatives thereof, may be isolated from the fermentation or cell culture and purified using any of a variety of conventional methods including: liquid chromatography such as normal or reversed phase, using HPLC, FPLC and the like; affinity chromatography (such as with inorganic ligands or monoclonal antibodies); size exclusion chromatography; immobilized metal chelate chromatography; gel electrophoresis; and the like. One of skill in the art may select the most appropriate isolation and purification techniques without departing from the scope of this invention.

In addition, the Sema-H polypeptides may be generated by any of several chemical techniques. For example, they may be prepared using the solid-phase synthetic technique originally described by R. B. Merrifield, "Solid Phase Peptide Synthesis. I. The Synthesis Of A Tetrapeptide", J. Am. Chem. Soc., 83, pp. 2149-54 (1963), or they may be prepared by synthesis in solution. A summary of peptide synthesis techniques may be found in E. Gross & H. J. Meinhofer, 4 The Peptides: Analysis, Synthesis, Biology; Modern Techniques Of Peptide And Amino Acid Analysis, John Wiley & Sons, (1981) and M. Bodanszky, Principles Of Peptide Synthesis, Springer-Verlag (1984).

Typically, these synthetic methods comprise the sequential addition of one or more amino acid residues to a growing peptide chain. Often peptide coupling agents are used to facilitate this reaction. For a recitation of peptide coupling agents suitable

for the uses described herein see M. Bodansky, *supra*. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different 5 protecting group is utilized for amino acids containing a reactive side group, e.g., lysine. A variety of protecting groups known in the field of peptide synthesis and recognized by conventional abbreviations therein, may be found in T. Greene, *Protective Groups* 10 *In Organic Synthesis*, Academic Press (1981).

In order to determine the presence of an active Sema-H polypeptide, functional assays or antibody recognition of specific epitopes can be used.

Therefore, in addition to the Sema-H 15 polypeptides, the invention also provides antibodies to Sema-H polypeptides. Such antibodies are immunoglobulin molecules or portions thereof that are immunologically reactive with a Sema-H polypeptides of the present invention. It should be understood that 20 the antibodies of this invention include antibodies immunologically reactive with fusion proteins and multimeric proteins comprising a Sema-H polypeptide. The generation of antibodies may be achieved by standard methods in the art for producing polyclonal 25 and monoclonal antibodies using a Sema-H polypeptide or fragment thereof as antigen. Such antibodies or active fragments thereof (such as Fab, Fab', F(ab)₂, fragments), may be used to assay *in vivo* or *in vitro* levels of Sema-H polypeptide. Increased *in vivo* levels of Sema-H 30 polypeptides may be indicative of the onset of metastasis. In a preferred embodiment such antibodies would be generated using fragments of semaphorin-H that

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represent unique portions of the molecule, as described previously.

An antibody of this invention may also be a hybrid molecule formed from immunoglobulin sequences 5 from different species (e.g., mouse and human) or from portions of immunoglobulin light and heavy chain sequences from the same species. It may be a molecule that has multiple binding specificities, such as a bifunctional antibody prepared by any one of a number 10 of techniques known to those of skill in the art including: the production of hybrid hybridomas; disulfide exchange; chemical cross-linking; addition of peptide linkers between two monoclonal antibodies; the introduction of two sets of immunoglobulin heavy and 15 light chains into a particular cell line; and so forth.

The antibodies of this invention may also be human monoclonal antibodies produced by any of the several methods known in the art. For example, human monoclonal antibodies may be produced by immortalized 20 human cells, by SCID-hu mice or other non-human animals capable of producing "human" antibodies, by the expression of cloned human immunoglobulin genes, by phage-display, or by any other method known in the art.

Alternatively, the genetic material of 25 antibody producing cells can be manipulated using techniques known to those of skill in the art. For example, immunoglobulin loci recovered from antibody producing cells can be manipulated to exchange the constant region for that of a different isotype or that 30 of a human antibody, or eliminated altogether. The variable regions can be linked to encode single chain F_v regions. Multiple F_v regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed.

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Once the genetic material is available, design of analogs which retain their ability to bind the desired target, as well as their human characteristics, is straightforward.

5 Once the appropriate genetic material is obtained and, if desired, modified to encode an analog, the coding sequences including those that encode, at a minimum, the variable regions of the heavy and light chain can be inserted into expression systems contained
10 on vectors which can be transfected into standard recombinant host cells. As described herein, a variety of such host cells can be used, however mammalian cells are preferred. Typical mammalian cell lines useful for this purpose include WHO cells, COS cells and 293
15 cells.

The production for the antibody is then undertaken by culturing the modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding
20 sequences. The antibodies are then recovered from the culture. The expression systems are preferably designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

25 In addition to the deliberate design of modified forms of the immunoglobulin genes to produce analogs, advantage can be taken of phage display techniques to provide libraries containing a repertoire of antibodies with varying affinities for the desired
30 antigen. For production of such repertoires, it is unnecessary to immortalize the B cells from the immunized animal; rather the primary B cells can be used directly as a source of DNA. The mixture of cDNAs obtained from B cells, e.g., derived from spleens, is

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used to prepare an expression library, for example, a phage display library transfected into *E. coli*. The resulting cells are tested for immunoreactivity to the desired antigen. Techniques for the identification of 5 high affinity human antibodies from such libraries are described by Griffiths, A.D. et al., EMBO J (1994) 13: 3245-3260; by Nissim, A., et al., *ibid*, 692-698, and by Griffiths, A.D., et al., *ibid*, 725-734. Ultimately, clones from the library are identified which produce 10 binding affinities of a desired magnitude for the antigen, and the DNA encoding the product responsible for such binding is recovered and manipulated for standard recombinant expression. Phage display libraries may also be constructed using previously 15 manipulated nucleotide sequences and screened in a similar fashion. In general, the cDNAs encoding heavy and light chain are independently supplied or are linked to form F_v analogs for production in the phage library.

20 The phage library is thus screened for the antibodies with the highest affinity for the antigen and the genetic material recovered from the appropriate clone. Further rounds of screening can increase the affinity of the original antibody isolated. The 25 manipulations described above for recombinant production of the antibody or modification to form a desired analog can then be employed.

In sum, one of skill in the art, provided with the teachings of this invention, has available a 30 variety of methods which may be used to alter the biological properties of the antibodies of this invention including methods which would increase or decrease the stability or half-life, immunogenicity, toxicity, affinity or yield of a given antibody

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molecule, or to alter it in any other way that may render it more suitable for a particular application.

Such antibodies may be monoclonal or polyclonal. Additionally, it is within the scope of 5 this invention to include second antibodies (monoclonal or polyclonal) directed to the anti-Sema-H antibodies. The present invention further contemplates use of these antibodies in a detection assay (immunoassay) for the Sema-H gene product.

10 The present invention further contemplates antibodies directed against mammalian, including rat, mouse and human Sema-H proteins or polypeptides. The antibodies of the invention may be generated by using the entire Sema-H protein as an antigen or by using 15 short peptides, encoding portions of the Sema-H protein, as antigens. When peptides are contemplated they have at least about 4 amino acids and preferably at least about 10 amino acids.

20 Preferably, specific peptides encoding unique portions of the mammalian Sema-H gene are synthesized for use as antigens for obtaining Sema-H antibodies.

25 Polyclonal antibodies directed against the Sema-H protein are prepared by injection of a suitable laboratory animal with an effective amount of the peptide or antigenic component, collecting serum from the animal, and isolating specific sera by any of the known immunoabsorbent techniques. Animals which can readily be used for producing polyclonal anti-Sema-H antibodies include chickens, mice, rabbits, rats, 30 goats, horses and the like. Chickens are preferred because a better immune response can be obtained and because antibodies can be collected from eggs rather than by bleeding. Although the polyclonal antibodies produced by this method are utilizable in virtually any

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type of immunoassay, they are generally less favored because of the potential heterogeneity of the product.

The use of monoclonal antibodies in the diagnostic or detection assays of the present invention 5 is particularly preferred because large quantities of antibodies, all of similar reactivity, may be produced. The preparation of hybridoma cell lines for monoclonal antibody production is done by fusing an immortal cell line and the antibody producing lymphocytes. This can 10 be done by techniques which are well known to those who are skilled in the art. (See, for example, Harlow, E. and Lane, D. Antibodies: A Laboratory Manual, Cold Spring Harbor Press, 1988; or Douillard, J.Y. and Hoffman, T., "Basic Facts About Hybridomas", in 15 Compendium of Immunology Vol. II, L. Schwartz (Ed.), 1981.

Unlike the preparation of polyclonal sera, the choice of animal for monoclonal antibody preparation is dependent on the availability of 20 appropriate immortal cell lines capable of fusing with the monoclonal antibody producing lymphocytes derived from the immunized animal. Mouse and rat have been the animals of choice for hybridoma technology and are preferably used. Humans can also be utilized as 25 sources for antibody producing lymphocytes if appropriate immortalized human (or nonhuman) cell lines are available. For the purpose of making the monoclonal antibodies of the present invention, the animal of choice may be injected with from about 0.01 30 mg to about 20 mg of the purified Sema-H antigen. Usually the injecting material is emulsified in Freund's complete adjuvant. Boosting injections are generally also required. The separate immortalized cell lines obtained by cell fusion may be tested for

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antibody production by testing the cell culture media for the ability to find the appropriate antigen.

Lymphocytes can be obtained by removing the spleen or lymph nodes of immunized animals in a sterile 5 fashion. Alternately, lymphocytes can be stimulated or immunized in vitro, as described, for example, in C. Reading, J. Immunol. Meth., 53: 261-291 (1982). To immortalize the monoclonal antibody producing lymphocytes, the lymphocytes must be fused to 10 immortalized cells. A number of cell lines suitable for fusion have been developed, and the choice of any particular line for hybridization protocols is directed by any one of a number of criteria such as speed, uniformity of growth characteristics, deficiency of its 15 metabolism for a component of the growth medium, and potential for good fusion frequency. Intraspecies hybrids, particularly between like strains, work better than interspecies fusions.

Several cell lines are available, including 20 mutants selected for the loss of ability to create myeloma immunoglobulin. Included among these are the following mouse myeloma lines: MPC-X45-6TG, P3 NS1/1-Ag4-1, P3-X63-Agl4 (all BALB/C derived), Y3'Agl.2.3 (rat), and U266 (human).

25 Cell fusion can be induced either by virus, such as Epstein-Barr or Sendai virus, or polyethylene glycol. Polyethylene glycol (PEG) is the most efficacious agent for the fusion of mammalian somatic cells. PEG itself may be toxic for cells, and various 30 concentrations should be tested for effects on viability before attempting fusion. The molecular weight range of PEG may be varied from 1,000 to 6,000. It give best results when diluted to from about 20% to about 70% w/w in saline or serum-free medium. Exposure

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to PEG at 37°C for about 30 seconds is preferred in the present case, utilizing murine cells. Extremes of temperature (i.e. about 45°C) are avoided, and preincubation of each component of the fusion system at 5 37°C prior to fusion gives optimum results. The ratio between lymphocytes and immortalized cells optimized to avoid cell fusion amongst lymphocytes ranges of from about 1:1 to about 1:10.

The successfully fused cells can be separated 10 from the immortalized cell line by any technique known by the art. The most common and preferred method is to choose an immortalized cell line which is Hypoxanthine Guanine Phosphoribosyl Transferase (HGPRT) deficient. Since these cells will not grow in an aminopterin- 15 containing medium, only hybrids of lymphocytes and immortalized cells will grow. The aminopterin containing medium is generally composed of hypoxanthine 1×10^{-4} M, aminopterin 1×10^{-5} M, and thymidine 3×10^{-5} M, commonly known as the HAT medium. Fused cells 20 are generally grown for two weeks and then fed with either regular culture medium or hypoxanthine, thymidine- containing medium.

The fused cell colonies are then tested for the presence of antibodies that recognize the Sema-H 25 protein. Detection of hybridoma antibodies can be performed using an assay where the antigen is bound to a solid support and allowed to react to hybridoma supernatants containing putative antibodies. The presence of antibodies may be detected by "sandwich" 30 techniques using a variety of indicators. Most of the common methods are sufficiently sensitive for use in the range of antibody concentrations secreted during hybrid growth.

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Cloning of hybrid cells can be carried out after 20-25 days of cell growth in selected medium. Cloning can be performed by cell limiting dilution in fluid phase or by directly selecting single cells 5 growing in semi-solid agarose. For limiting dilution, cell suspensions are diluted serially to yield a statistical probability of having only one cell per well. For the agarose techniques, hybrids are seeded in a semisolid upper layer, over a lower layer 10 containing feeder cells. The colonies from the upper layer may be picked up and eventually transferred to wells.

Antibody-secreting hybrid cells can be grown in various tissue culture flasks, yielding supernatants 15 with variable concentrations of antibodies. In order to obtain higher concentrations, hybrid cells may be transferred into animals to obtain inflammatory ascites. Antibody- containing ascites can be harvested 8-12 days after intraperitoneal injection. The ascites 20 contain a higher concentration of antibodies but include both monoclonals and immunoglobulins from the inflammatory ascites. Antibody purification may then be achieved by, for example, affinity chromatography.

Finally, Sema-H polypeptide antibodies may be 25 employed, for example, to purify Sema-H polypeptides from either native sources or transformed hosts expressing Sema-H polypeptides. For example, the antibodies can be conjugated by standard techniques to an insoluble matrix to form an immunoaffinity resin.

30 The present invention further provides methods for diagnosing metastatic cancer and for distinguishing metastatic tumors from benign tumors. The data presented herein demonstrates that Sema-H expression is higher in metastatic tumor cells than in

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normal or non-metastatic tissue. Such metastatic tumors could be derived from the lung, liver, kidney, mammary gland, epithelial, thyroid, leukemic, pancreatic, endometrial, ovarian, cervical, skin, colon 5 or lymphoid tissue and compared to benign tumor cells or the corresponding normal cells. Accordingly, in one aspect of the invention, metastatic cancer can be detected in patient's serum by a simple immunoassay. Moreover, metastatic cancer can also be diagnosed in 10 tissue biopsies by the present immunoassays or by in situ hybridization assays.

In accordance with the present inventive discovery, the increased expression of the Sema-H gene in a cell or tissue is strongly indicative of 15 metastatic potential. The present invention utilizes this unexpected and surprising correlation of high mammalian Sema-H gene expression with high metastatic potential to detect or diagnose malignant cancer. Both the mammalian Sema-H nucleic acid and antibodies 20 directed against mammalian Sema-H proteins are contemplated for the diagnosis of malignant cancer.

A nucleic acid probe of the present invention may be any portion or region of a mammalian Sema-H RNA or DNA sufficient to give a detectable signal when 25 hybridized to Sema-H mRNA derived from a tissue sample. The nucleic acid probe produces a detectable signal because it is labeled in some way, for example because the probe was made by incorporation of nucleotides linked to a "reporter molecule".

30 A "reporter molecule", as used herein, means any molecule which, by its chemical nature, provides an analytically identifiable signal allowing detection of the hybridized probe. Detection may be either quantitative or non-quantitative. One of skill in the

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art will recognize that although any reporter molecule known in the art can be used, the most commonly used reporter molecules in this type of assay are enzymes, fluorophores and radionuclides. Such reporter 5 molecules typically are covalently linked to nucleotides which are incorporated into a Sema-H DNA or RNA. Examples of commonly used enzymes include horseradish peroxidase, alkaline phosphatase, glucose oxidase and β -galactosidase. The substrates to be used 10 with the specific enzymes are generally chosen for the production, upon hydrolysis by the corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for horseradish peroxidase, 15 1,2-phenylenediamine, 5-aminonalicyclic acid or toluidine are commonly used.

Examples of fluorescent labeling agents are fluorescein isocyanate (FIC), fluorescein isothiocyanate (FITC), and the like. Examples of 20 radioactive element are ^{125}I or ^{51}Cr which produce gamma ray emissions, or a radioactive element that emits positrons which produce gamma rays upon encounters with electrons present in the test solution, such as ^{11}C , ^{15}O , or ^{13}N . Detection may also be by other methods, 25 for example via avidin-biotin complexes.

Incorporation into a Sema-H probe may be by 30 nick translation, random oligo priming, by 3' or 5' end labeling, by labeled single-stranded DNA probes using single-stranded bacteriophage vectors (e.g. M13 and related phage), or by other means known in the art, (Sambrook *et al.*, 1989, Molecular Cloning, A laboratory Manual. Cold Spring Harbor Laboratory Press. Pages 10.1-10.70). Incorporation of a reporter molecule into a Sema-H RNA probe may be by synthesis of Sema-H RNA

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using T3, T7, Sp6 or other RNA polymerases (Sambrook *et al.*, *supra*: 10.27-10.37).

Detection or diagnosis of metastatic cancer by the nucleic acid probe of the present invention can 5 be by a variety of hybridization-techniques which are well known in the art. In one embodiment, patient tissue specimens are sectioned and placed onto a standard microscope slide, then fixed with an appropriate fixative. The Sema-H RNA or DNA probe, 10 labeled by one of the techniques described above, is added. The slide is then incubated at a suitable hybridization temperature (generally 37°C to 55°C) for 1-20 hours. Non-hybridized RNA or DNA probe is then removed by extensive, gentle washing. If a non- 15 radioactive reporter molecule is employed in the probe, the suitable substrate is applied and the slide incubated at an appropriate temperature for a time appropriate to allow a detectable color signal to appear as the slide is visualized under light 20 microscopy. Alternatively, if the Sema-H probe is labeled radioactively, slides may be dipped in photoemulsion after hybridization and washing, and the signal detected under light microscopy after several days, as exposed silver grains.

25 In another embodiment, metastatic cancer is detected from RNA derived from tissue specimens by the Sema-H nucleic acid probe. RNA is isolated using methods known in the art and from specimens fixed onto nitrocellulose or nylon filters. Specimen mRNA can be 30 purified, or specimen cells may be simply lysed and cellular mRNA fixed unto a filter. Specimen mRNA can be size fractionated through a gel before fixation onto a filter, or simply dot blotted unto a filter. Sema-H gene expression is then detected using probes of the

invention and hybridization techniques that are well known in the art.

In a further embodiment, a kit for the detection of Sema-H mRNA is provided. In general, a 5 kit for detection of Sema-H mRNA contains at least one Sema-H nucleic acid of the invention. Such a Sema-H nucleic acid probe may be labeled with a reporter molecule or unlabeled. The kit may include an unlabeled Sema-H nucleic acid which can be modified by 10 the kit user to include a reporter molecule, for example by nick translation or RNA transcription.

In a further embodiment, such a kit would be directed towards the quantitation of Sema-H mRNA in a cell using techniques known to those of skill in the 15 art, for example, Northern Blot analysis or RT-PCR. Quantification of Sema-H mRNA based on appropriate standards, easily determined by one of skill in the art using the disclosed Sema-H DNA sequence, would distinguish metastatic and potentially metastatic cells 20 from non-metastatic cells.

In another embodiment, the kit is compartmentalized: a first container can contain Sema-H RNA at a known concentration to act as a standard or positive control, a second container can contain Sema-H 25 DNA suitable for synthesis of a detectable nucleic acid probe, and a third and a fourth container can contain reagents and enzymes suitable for preparing said Sema-H detectable probe. If the detectable nucleic acid probe is made by incorporation of an enzyme reporter 30 molecule, additional containers can contain a substrate, or substrates, for the enzyme provided.

Another embodiment of the present invention provides a method for diagnosing metastatic cancer by contacting a biological sample taken from an individual

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to be tested for metastatic cancer with an antibody of the invention. Detection of an antigen-antibody complex in this immunoassay is diagnostic of metastatic cancer. According to this embodiment, the antibody of 5 the invention can further be brought into contact with a specimen of bodily fluids, bodily secretions or tissue from an individual to be tested for the presence of the semaphorin-H antigen under conditions sufficient to form an antibody-antigen complex. The body fluids 10 are, for example, blood and urine. The bodily secretions are, for example, urine, tears, sweat, saliva, cervical secretions, vaginal secretions, mucosal secretions or intraperitoneal ascitic fluid. The tissue can be, but does not have to be, excised 15 from the individual.

In a preferred embodiment, the present invention provides a method for diagnosing metastatic cancer which involves contacting a sample from an individual to be tested for such cancer with an 20 antibody reactive with a mammalian Sema-H protein or an antigenic fragment thereof, for a time and under conditions sufficient to form an antigen-antibody complex, and detecting the antigen-antibody complex.

The presence of the Sema-H-protein, or its 25 antigenic components, in a patient's serum, tissue or biopsy sample can be detected utilizing antibodies prepared as above, either monoclonal or polyclonal, in virtually any type of immunoassay. A wide range of immunoassay techniques are available as can be seen by 30 reference to Harlow, et al. (Antibodies: A Laboratory Manual, Cold Spring Harbor Press, 1988) and U.S. Patent No. 4,016,043 and 4,424,279. This, of course, includes both single-site and two-site, or "sandwich" of the noncompetitive types, as well as in traditional

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competitive binding assays. Sandwich assays are among the most useful and commonly used assays. A number of variations of the sandwich assay technique exist, and all are intended to be encompassed by the present invention. Briefly, in a typical forward assay, an unlabeled antibody is immobilized in a solid substrate and the sample to be tested brought into contact with the bound molecule. After a suitable period of incubation, for a period of time sufficient to allow formation of an antibody-antigen binary complex, a second antibody, labeled with a reporter molecule capable of producing a detectable signal is then added and incubated, allowing time sufficient for the formation of a ternary complex of antibody-labeled antibody. Any reacted material is washed away, and the presence of the antigen is determined by observation of a signal produced by the reporter molecule. The results may either be qualitative, by simple observation of the visible signal, or may be quantitated by comparing with a control sample containing known amounts of hapten. Variations on the forward assay include a simultaneous assay, in which both sample and labeled antibody are added simultaneously to the bound antibody, or a reverse assay in which the labeled antibody and sample to be tested are first combined, incubated and then added to the unlabeled surface bound antibody. These techniques are well known to those skilled in the art, and then possibly of minor variations will be readily apparent. As used herein, "sandwich assay" is intended to encompass all variations on the basic two-site technique.

The Sema-H protein may also be detected by a competitive binding assay in which a limiting amount of

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antibody specific for the Sema-H protein is combined with specified volumes of samples containing an unknown amount of the Sema-H protein and a solution containing a detectably labeled known amount of the Sema-H
5 protein. Labeled and unlabeled molecules then compete for the available binding sites on the antibody. Phase separation of the free and antibody-bound molecules allows measurement of the amount of label present in each phase, thus indicating the amount of antigen or
10 hapten in the sample being tested. A number of variations in this general competitive binding assays currently exist.

In any of the known immunoassays, for practical purposes, one of the antibodies or the
15 antigen will be typically bound to a solid phase and a second molecule, either the second antibody in a sandwich assay, or, in a competitive assay, the known amount of antigen, will bear a detectable label or reporter molecule in order to allow visual detection of
20 an antibody-antigen reaction. When two antibodies are employed, as in the sandwich assay, it is only necessary that one of the antibodies be specific for the Sema-H protein or its antigenic components. The following description will relate to a discussion of a
25 typical forward sandwich assay; however, the general techniques are to be understood as being applicable to any of the contemplated immunoassays.

In the typical forward sandwich assay, a first antibody having specificity for the Sema-H
30 protein or its antigenic components is either covalently or passively bound to a solid surface. The solid surface is typically glass or a polymer, the most commonly used polymers being cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or

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polypropylene. The solid supports may be in the form of tubes, beads, discs or microplates, or any other surface suitable for conducting an immunoassay. The binding processes are well-known in the art and

5 generally consisting of crosslinking covalently binding or physically adsorbing the molecule to the insoluble carrier. Following binding, the polymer-antibody complex is washed in preparation for the test sample. An aliquot of the sample to be tested is then added to

10 the solid phase complex and incubated at a suitable temperature ranging from about 4°C to about 37°C (for example 25°C) for a period of time sufficient to allow binding of any subunit present in the antibody. The incubation period will vary but will generally be in

15 the range of about 2-40 minutes to several hours. Following the incubation period, the antibody subunit solid phase is washed and dried and incubated with a second antibody specific for a portion of a Sema-H hapten. The second antibody is linked to a reporter

20 molecule which is used to indicate the binding of the second antibody to the hapten.

The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules. In the case of an

25 enzyme immunoassay, an enzyme is conjugated to the second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled

30 artisan. Commonly used enzymes include horseradish peroxidase, glucose oxidase, β -galactosidase and alkaline phosphates, among others. The substrates to be used with the specific enzymes are generally chosen for the production, upon hydrolysis by the

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corresponding enzyme, of a detectable color change. For example, p-nitrophenyl phosphate is suitable for use with alkaline phosphatase conjugates; for peroxidase conjugates, 1,2-phenylenediamine, 5-5 aminosalicylic acid, or toluidine are commonly used. It is also possible to employ fluorogenic substrates, which yield a fluorescent product rather than the chromogenic substrates noted above. In all cases, the enzyme-labeled antibody is added to the first antibody 10 hapten complex, allowed to bind, and then the excess reagent is washed away. A solution containing the appropriate substrate is then added to the ternary complex of antibody-antigen-antibody. The substrate will react with the enzyme linked-to the second 15 antibody, giving a qualitative visual signal, which may be further quantitated, usually spectrophotometrically, to give an indication of the amount of hapten which was present in the sample.

Alternately, fluorescent compounds, such as 20 fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody absorbs the light energy, inducing a state of 25 excitability in the molecule, followed by emission of the light at a characteristic color visually detectable with a light microscope. The fluorescent labeled antibody is allowed to bind to the first antibody-hapten complex. After washing off the unbound reagent, 30 the remaining ternary complex is then exposed to the light of the appropriate wavelength, the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence techniques are very well established in the art. However, other reporter

molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. It will be readily apparent to the skilled technician how to vary the procedure to suit the required purpose.

5 In another embodiment, the antibodies directed against the Sema-H protein may be incorporated into a kit for the detection of the Sema-H protein. Such a kit may encompass any of the detection systems contemplated and described herein, and may employ

10 either polyclonal or monoclonal antibodies directed against the Sema-H protein. Both Sema-H antibodies complexed to a solid surface described above or soluble Sema-H antibodies are contemplated for use in a detection kit. A kit of the present invention has at

15 least one container having an antibody reactive with a mammalian Sema-H polypeptide. However, the present kits can have other components. For example, the kit can be compartmentalized: the first container contains Sema-H protein as a solution, or bound to a solid

20 surface, to act as a standard or positive control, the second container contains anti-Sema-H primary antibodies either free in solution or bound to a solid surface, a third container contains a solution of secondary antibodies covalently bound to a reporter

25 molecule which are reactive against either the primary antibodies or against a portion of the Sema-H protein not reactive with the primary antibody. A fourth and fifth container contains a substrate, or reagent, appropriate for visualization of the reporter molecule.

30 The kit of this embodiment could be further designed to perform techniques known to those of skill in the art, such as immunoblotting, dot blotting and Western assays using the antibodies of the present invention.

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Quantification of Sema-H polypeptide based upon appropriate standards known to those of skill in the art is used for distinguishing metastatic and potentially metastatic cells from non-metastatic cells.

5 The subject invention therefore encompasses polyclonal and monoclonal antibodies useful for the detection of Sema-H protein as a means of diagnosing metastatic cancer. Said antibodies may be prepared as described above, then purified, and the detection 10 systems contemplated and described herein employed to implement the subject invention.

The present invention also contemplates treating metastatic cancers and tumors by inactivating, destroying or nullifying the Sema-H gene or protein, or 15 cells expressing the Sema-H gene. The treatment of cancer, as described in the specification and claims, contemplates preferably lung, liver, kidney, thyroid, mammary gland, leukemic, pancreatic, endometrial, ovarian, cervical, skin, colon or lymphoid cancers.

20 A preferred use then, according to this embodiment, is in the treatment of cancer in a patient. The term "patient", as used herein refers to any mammal, especially humans. DNA sequences encoding inactive Sema-H polypeptides would be useful in gene 25 therapy to inhibit the metastatic potential of the cell. According to this embodiment, the inactive Sema-H polypeptide encoded by this DNA would compete for substrate with native, active Sema-H. This would decrease the Sema-H activity in the cell, inhibiting 30 metastasis. Preferred cells to be treated by this method are cancer cells.

In another embodiment, antibodies, prepared as described above, may be utilized to direct toxic events to Sema-H protein expressing cells. Either

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unconjugated anti-sema-H antibodies or anti-Sema-H antibodies conjugated to a therapeutic molecule may be employed in the therapy of cancer. For example, it may be advantageous to couple the antibodies of this 5 invention to toxins such as diphtheria, pseudomonas exotoxin, ricin A chain, gelonin, etc., or antibiotics such as penicillins, tetracyclines and chloramphenicol.

Moreover, the present invention provides a method of inhibiting metastasis in a cancer cell by 10 introducing into the cancerous cell an anti-sense nucleic acid sequence. Antisense Sema-H nucleic acids can inhibit metastatic cancer by binding to sense Sema-H mRNA. Such binding can either prevent translation of Sema-H protein or destroy Sema-H sense mRNA, e.g., 15 through the action of RNaseH. Accordingly, less Sema-H protein is available to potential metastatic tumor cells and metastasis of these cells is inhibited. In one embodiment, such an antisense nucleic acid is a DNA or RNA molecule having at least 10 nucleotides of the 20 antisense strand of [SEQ ID NO:1]. Preferably, the antisense Sema-H nucleic acids of the present invention have at least 15 or 17 nucleotides.

In one embodiment, this method employs an expression vector including a nucleic acid encoding an 25 antisense nucleotide sequence for Sema-H operably linked to a segment of the vector which can effect expression of an antisense Sema-H RNA. Any of the foregoing expression vectors which can express high levels of Sema-H RNA can be used for this method.

30 Another embodiment of the present invention provides pharmaceutical compositions comprising an antibody reactive with a mammalian Sema-H polypeptide or fragment or derivative thereof, an antisense Sema-H nucleic acid or a Sema-H protein.

The active ingredients of a pharmaceutical composition containing the Sema-H protein (i.e. pro-metastatic reagent) or anti-Sema-H antibodies and antisense Sema-H nucleic acids (i.e. anticancer reagents) are contemplated to exhibit effective therapeutic activity, for example, in treating cancer. Thus the active ingredients of the therapeutic compositions containing Sema-H protein anti-cancer reagents, are administered in therapeutic amounts which 5 depend on the particular disease, route of administration, and other factors well known to those of skill in the art. Preferably, from about 0.5 µg to about 2000 mg per kilogram of body weight per day may be administered. The dosage regimen may be adjusted to 10 provide the optimum therapeutic response. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. The active compound may be administered in a convenient manner 15 such as by the oral, intravenous (where water soluble), intramuscular, subcutaneous, intranasal, intradermal or suppository routes. Depending on the route of administration, the active ingredients may be required to be coated in a material to protect said ingredients 20 from the action of enzymes, acids and other natural conditions which may inactivate said ingredients. In order to administer Sema-H protein or anti-cancer reagents of this invention by other than parenteral administration, they should preferably be coated by, or 25 administered with, a material to prevent its inactivation. For example, Sema-H protein or anti-cancer reagents may be administered in an adjuvant, co-administered with enzyme inhibitors or in liposomes. 30 Adjuvants useful for this purpose include resorcinols,

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nonionic surfactants such as polyoxyethylene oleyl ether and n-hexadecyl polyethylene ether. Enzyme inhibitors include pancreatic trypain inhibitor, diisopropylfluorophosphate (DFP) and trasylol.

5 Liposomes include water-in-oil-in-water P40 emulsions as well as conventional liposomes.

The anti-Sema-H proteins, antibodies, or anti-Sema-H nucleic acids may also be administered parenterally or intraperitoneally. Dispersions can 10 also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof, and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

15 The pharmaceutical forms suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. In all cases the form must be 20 sterile and must be fluid to the extent that easy syringe ability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier 25 can be a solvent or dispersion medium containing, for example, water, ethanol, pall (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by 30 the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The preventions of the action of microorganisms can be brought about by various antibacterial and antifungal

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agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged 5 absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by 10 incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various 15 sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of 20 preparation are vacuum-drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

When the Sema-H protein or anti-cancer 25 reagents are suitably protected as described above, the active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into 30 tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, trochee, capsules, elixirs, suspensions,

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syrups, wafers, and the like. Compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 0.5 μ g and 2000 μ g of active compound.

5 The tablets, troches, pills, capsules, and the like, as described above, may also contain the following: a binder such as gum tragacanth, acacia corn starch or gelatin; excipients such as dicalcium phosphate; a disintegrating agent such as corn starch, 10 potato starch, alginic acid, and the like; a lubricant such as magnesium stearate; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil or wintergreen or cherry flavoring. When the dosage unit form is a 15 capsule it may contain, in addition to materials of the above type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills or capsules may be coated with shellac, 20 sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form 25 should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

It is especially advantageous to formulate 30 parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined

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quantity of the active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are
5 dictated by and directly depending on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such as active material for the treatment of disease in
10 living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principal active ingredient is compounded for convenient and effective administration in
15 effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principal active compound in amounts ranging from 0.5 μ g to about 2000 μ g. Expressed in
20 proportions, the active compound is generally present in from about 10 μ g to about 2000 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of
25 administration of the said ingredients.

As used herein "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and adsorption delaying agents, and the like.
30 The use of such media gents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, use thereof in the therapeutic compositions is contemplated. Supplementary active

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ingredients can also be incorporated into the compositions.

The Sema-H DNA sequences of this invention, of fragments thereof, may be used to identify 5 homologous DNA sequences so as to potentially identify other members of this family.

These DNA sequences, or fragments thereof, are useful in a number of therapeutic and diagnostic applications. According to one embodiment, the DNA 10 sequences encoding a Sema-H polypeptide may be used in either *in vitro* or *in vivo* gene therapy. In this embodiment, the DNA must be contained in a suitable vehicle for gene therapy. Such vehicles are known in the art. For example, various viruses that are capable 15 of transferring genetic material to a target cell, without replicating in that cell have been described (United States patents 5,112,767, 5,240,846 and 5,112,767, the disclosures of which are herein incorporated by references). Such viruses include 20 replication-defective adenoviruses, adeno-associated viruses (AAV), and replication defective retroviruses, such as PLJ, pZip, pWe and pEM.

Gene delivery systems other than viruses can also be employed in the methods of this invention. For 25 example, the gene to be transferred may be packaged in a liposome. When cells are incubated with DNA-encapsidated liposomes, they take up the DNA and express it. To form these liposomes, one mixes the DNA of an expression vector which expresses the gene to be 30 transferred with lipid, such as N-[1-(2,3-dioleyloxy)propyl]-N,N,N-tri-methylammonium chloride (DOTMA) in a suitable buffer, such as H_{epes} buffered saline. This causes the spontaneous formation of lipid-DNA complexes (liposomes) which can be

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employed in the methods of this invention [P. L. Felgner et al., Proc. Natl. Acad. Sci. USA, 84, pp. 7413-17 (1987)].

Another gene delivery system that may be 5 utilized in this invention is DNA-protein complexes. The formation of these complexes is described in United States patent 5,166,320, the disclosure of which is herein incorporated by reference. Specifically, these complexes comprise the gene to be transferred (together 10 with promoter, enhancer sequences and other DNA necessary for expression in the target cell) linked via a suitable polymer, such as polylysine, to a polypeptide ligand for a receptor or other cell surface protein. This complex is taken up by the target cells 15 via endocytosis after the ligand binds to the cell surface receptor. The DNA is then cleaved from the rest of the complex via intracellular enzymes which cut the polymer linker. Such complexes are particularly useful in targeting specific cell types. For examples, 20 cancer cells may be specifically targeted through the use of proteins which specifically recognize tumor cell surface markers or receptors.

Antibodies to Sema-H polypeptides also have potential therapeutic use in preventing metastasis in a 25 patient. Such antibodies may be directed to their target cells through conjugation to cell-specific molecules. The conjugation of such antibodies and the identity of cell-specific targeting molecules is known in the art.

30 Another embodiment of the present invention relates to the animal tumors and tumor cell lines developed in accordance with the present invention which are useful as model systems of the metastatic process. These tumors and cell lines can be utilized

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for screening anti-metastatic drugs and for developing therapeutic regimens for the treatment of malignant cancer is provided by the present invention.

The tumors or cell lines of the present invention each would have a highly predictable metastatic potential; however the metastatic potentials of related, but separate, tumors or cell lines can be very different. These tumors and cell lines are useful for the development of a variety of human cancer therapies, for several reasons. First, cancer cells all have similar properties, including, for example, unrestrained growth and lack of contact inhibition, which suggests that the process of cancer development is similar in all cancers. Second, the morphologies and biochemical properties of the tumors developed after injection of these tumor-derived cells are identical to analogous tumors in humans. Hence, potential anti-cancer therapies or drugs may effectively be screened by employing the animal model system of the current invention.

The utility of these unique tumors and cell lines is apparent to one skilled in the art. Briefly, animals are injected with tumors or tumor-derived cells which have a predictable metastatic potential. A proportion of the animals are treated with a potential anti-cancer drug or therapy. After a suitable period of time, all animals are sacrificed and the tissues of both treated and non-treated animals are examined for the development of primary and secondary (metastatic) tumors. If a therapeutic regimen is successful, the treated animals should have a much lower incidence of tumor formation.

Both mouse and rat model systems are provided by the present invention for the development of cancer

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therapy. This is done by intramuscular transplantation or subcutaneous tail transplantations of the original spontaneous mammary tumor cells into syngeneic mice. Intramuscular transplantation has yielded a cell line 5 called CSML-0 which has low metastatic potential. Solitary lung metastasis are detected in less than 10% of CSML-0 injected animals sacrificed because of a moribund condition. The highly metastatic CSML-100 cell line has been generated by selection of the 10 metastatic phenotype through successive subcutaneous transplantations of CSML metastatic cells into the tail. The CSML-50 cell line, selected during the generation of CSML-100, has an intermediate level of metastatic potential.

15 A variety of rat tumors have been generated by irradiating normal Fischer 344 rat thyroid cell suspensions and then transplanting these cells into rats. Grafts of non-irradiated thyroid cells develop into morphologically and functionally normal thyroid 20 tissue after transplantation into Fischer 344 syngeneic rats, if elevated levels of thyroid stimulating hormone are also provided. Irradiation of thyroid cell suspensions before transplantation has produced a series of rat thyroid carcinomas which are 25 histopathologically identical to human counterparts.

The extensive variety of tumors and cell lines, and the varying metastatic potential of these tumors and cell lines, provides mouse and rat model systems amenable to carefully controlled studies 30 directed towards the dissection of the metastatic process. Therapeutic regimens for treatment of malignant cancer can be developed by controlled studies of groups of animals injected with cells of high, low and intermediate metastatic potential. A drug, or

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pharmaceutical composition suspected of having anti-metastatic potential, may be used to treat a proportion of animals from each group. The incidence of metastasis amongst the animals receiving the drug or 5 pharmaceutical composition may be compared with the incidence amongst animals not receiving treatment. Therefore, the present invention provides an animal system for distinguishing effective anti-metastatic drugs and therapies from those that are ineffective.

10 In order that the invention described herein may be more fully understood, the following examples are set forth. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting this invention in any 15 manner.

Example 1 - Cloning of Mouse Semaphorin-H and Correlation of SemaH Expression to Metastasis

20 A. Identification of Semaphorin Transcripts in Metastatic Cell Lines

Differential display was performed to compare the mRNA populations of two metastatic cell lines 66c14 and 4T1 with non-metastatic cell line 67NR. Total RNA 25 of confluent cultures of the 4T1, 66c14 and 67NR cell lines was DNase-treated. The mRNA with UC as the final 3'-nucleotides were reverse transcribed using 5'T₁₁AG-3' primer and PCR was subsequently performed using the same primer in combination with a number of 10mer 30 primers. The sequence of the 10mer primers is described in (17). Transcripts of the M-semaH gene were identified through the use of the 10mer primer 5'-GTTTTCGCAG-3' in the PCR amplifications. The protocol

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for reverse transcription and PCR was as described (2), with the following modifications: Superscript reverse transcriptase (Gibco BRL) was used in the reverse transcription, and in the PCR, Thermoprime plus Taq-5 polymerase and corresponding buffer was used with 1.0mM as the final concentration of MgCl₂ (Advanced Biotech).

PCR conditions were also modified and were 94°C, 30s; 40°C, 2min; 72°C, 30s, for 5 remaining cycles, then 94°C, 30s; 42°C, 2min; 72°C, 30s for 35 10 cycles followed by 72°C, 5min. The remaining steps of electrophoresis, extraction of bands, reamplification and cloning were as described (2).

An amplified 525 base pair fragment was identified in the metastatic cell lines but not the 15 non-metastatic cell lines and this fragment was extracted from a sequencing gel. The fragment was found to originate from a novel mouse semaphorin gene. Northern analysis was performed to demonstrate the differential expression of transcripts hybridizing to 20 the 525 bp fragment in metastatic cell lines. The probe hybridized to three transcripts of approximately 7.0 kb, 4.5 kb and 4.0 kb in the expressing cell lines.

Examination of the ability of the cells to form lung metastasis in an experimental and spontaneous 25 metastasis assay revealed a perfect correlation between the expression of M'semaH and the metastatic potential of the five cell lines in Figure 1B. Subsequent analysis of 14 additional cell lines identified a strong correlation between M-semaH transcripts and 30 metastasis (Figure 1C).

B. Cloning of M-SemaH DNA

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A 525 base pair fragment, amplified and extracted from 66cl4 or 4T1 metastatic cells after differential display analysis (as described above), was hybridized to a λ -cDNA library derived from the 5 metastatic CSML-100 cell line. The cDNA library was constructed from the CSML-100 cell line using the ZAP-cDNA synthesis kit and ligated into Uni-ZAP XR vectors using ZAP-cDNA Gigapack II Gold Cloning Kit (Stratagene) as would be known to one of skill in the 10 art. The cDNA fragments identified through hybridization were examined by PCR and restriction analysis, after which two fragments of 4435 base pairs and 3675 base pairs were sequenced.

The 3675 base pair fragment was found to be 15 lacking a region of 478 base pairs localized 169 base pairs from the 3'-end (Figure 2A). Northern analysis with a probe specific for the 478 base pair (probe C, Figure 2C) detected only the 4.5 kb and 7.0 kb transcripts (compare probe B, Figure 2B with probe C, 20 Figure 2C). The 3675 base pair cDNA therefore corresponds to the 4.0 kb transcript. To describe its variant nature we designated it M-semaH-v (SEQ ID NO:2)

5' RACE analysis was performed using the 25 Marathon cDNA cloning kit (Clontech). First strand cDNA was synthesized using 1mM of oligo(dT) primer (from kit) and 1 μ g of poly A⁺-RNA isolated from metastatic cell line 66cl14. Primers specific to M-semaH were 5'CTCTTAGGACCACCTGTTCAC-3' and 5'-GGGGTTGAGGAGGAAACA-3' (DNA Technology, Denmark). Other 30 components and instructions as provided by the manufacturer. RACE analysis demonstrated shared 5' ends between the two transcripts. Northern analysis with a probe specific to the 5'untranslated portion of the 4435 bp fragment hybridized to both the 4.0 and

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4.5kb transcripts (Figure 2D). Those of skill in the art will appreciate that DNA encoding additional mammalian sema-H can be cloned using these methods.

5 Example 2 - Expression of M-semaH Polypeptides

A. Expression in Prokaryotes

The mouse semaH open reading frame (ORF) excluding the sequence encoding the eukaryote signal peptide is cloned by PCR from the 4430 bp semaH cDNA, 10 which is previously cloned in a pBluescript SK phagemid (vector obtained by Stratagene). The sense primer is 5'-AAACACGCATGCGAGCCCTCCTACGCCAG-3' encoding a *SphI* site and the SemaH sequence PSYAR; and the antisense primer is 5'-CCCCCCGGGGGTCAAGGAGAGCAGCG-3' which 15 encodes a *XmaI* site and is complementary to the sequence encoding the C-terminal part of SemaH. The PCR fragment is subcloned into the pCR2.1 vector using the TOPO-TA cloning kit (Invitrogen). Transformants are selected for ampicillin resistance. Plasmids are 20 purified from selected colonies, restricted with *SphI* (Pharmacia) and *XmaI* enzymes (New England Biolabs), released fragments gel purified using the QIAQuick Gel Extraction Kit (Qiagen) and cloned into the *SphI* and *XmaI* sites of the pQE30 vector (Qiagen) next to a N- 25 terminal His tag encoded by this vector. This will generate the pQE30/Mouse-His construct for protein expression in M15 bacteria (Qiagen), see below.

B. Expression in Mammalian Cells

To express the Mouse SemaH in mouse tumor 30 cell lines we use the expression vector pcDNA3.1/ (-),

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which carries resistance to the antibiotic Zeocin (Invitrogen). The ORF of M-semaH is in one of two forms under the control of the CMV-promoter: 1) the ORF with the native UGA stop codon (pcDNA3.1/SemaH/zeo^R) and, 2) the ORF modified in the three last amino acids to allow the C-terminal fusion with the taq sequence NH₂-LVPRGSGPEQKLISEEDLNSAVDHHHHHHH-COOH, comprising a Trombin cleavage site, a Myc epitope and a Histidin taq (pcDNA3.1/SemaH-TMH/zeo^R). In both cases the 10 pBluescript SK phagemid containing the 4430 bp cDNA of the 5.5 kb semaH transcript is used as template for the PCR reactions.

The sense primer 5'-

AGAGGAGGGCCCGCCGCCACCATGGCACC-3' encoding an *ApaI* site 15 and a consensus Kozak sequence next to the native SemaH N-terminal sequence MAP will be used together with the antisense primer 5'-CGGCAGAGGGGGCCCTCAGGAGAGCAGCG-3' encoding an *ApaI* site complementary to the sequence encoding the native SemaH C-terminal sequence TLLS- 20 stop. The fragment is subcloned into the pCR2.1 vector using the TOPO-TA cloning Kit (Invitrogen). Transformants are selected for Ampicillin resistance. Plasmids purified from selected colonies are restricted 25 with *ApaI* enzyme (Pharmacia), released fragments gel purified using the QiaQuick Gel Extraction Kit (Qiagen) and cloned into the *ApaI* site of the vector pcDNA3.1(-)/zeo^R (Invitrogen). The resulting construct is transformed into TOP10F' bacteria (Invitrogen) and transformants are selected for resistance to Zeocin 30 (Invitrogen).

The same sense primer as above is used together with the antisense primer 5'-

GCCGGGGGCCGCTCCCTGGGGGACCAGCGTGTG-3' encoding an *ApaI*

site and being complementary to the native SemaH C-terminal sequence encoding HTL, skipping the stop codon and instead fused to the peptide VPRGCGP comprising a thrombin cleavage site. The fragment is subcloned as 5 described above and after identical procedures cloned into the *ApaI* site of the pcDNA3.1(-)/Mye-HisB/neor^R vector (Invitrogen). The plasmid is transformed into TOP10F' bacteria (Invitrogen) and transformants are selected for ampicillin resistance. The C-terminal 10 part of SemaH is fused this way to the vector encoded Mye epitope and His Tag. Plasmids, purified from selected colonies, are restricted with *PmeI* enzyme (New England Biolabs) cutting on either site of the entire semaH-TMH fragment, and cloned into the *PmeI* site of 15 the pcDNA3.1(-)/zeo^R (Invitrogen) to generate a pcDNA3.1/SemaH-TMH/zeo^R construct. Transformants are obtained and scored as above. The inserted semaH fragment in the pcDNA3.1/SemaH-TMH construct will be used as template in a PCR amplification using the sense 20 primer 5'-GGTCACTCTGCAGGCCCTCCTACGCCAG-3', encoding a *PstI* site and the amino acid sequence PSYAR which follows the native N-terminal signal sequence of M-SemaH, and the antisense primer 5'-GCCGGGCGGCCGCCCCTCGGGGG-3' specific to the binding site 25 of the antisense-primer used in constructing the pcDNA3.1/SemaH-TMH/zeo^R but encoding a *NotI* site in place of the *ApaI* site. The fragment is subcloned in a pCR2.1 vector as above, released by restriction with *NotI* and *PstI* enzymes (Pharmacia) and cloned into the 30 respective sites of a pPICZ \square B/zeo^R vector (Invitrogen) to generate a pPICZ \square B/SemaH-TMH/zeo^R construct. In this construct, the semaH ORF is sitting next to the *S. Cerevisiae* factor signal sequence under the control of

the *P. Pastoris* AOX1 gene promoter, which is inducible by methanol. In the C-terminal the semaH ORF is fused to a vector encoded peptide comprising a Mye epitope and a His tag similar to the one in pcDNA3.1/SemaH-TMH/zeo^R construct. Transformants are obtained and scored as above.

Example 3 - Cloning of the human SemaH homologue

Cloning of the human homologue of semaH is performed by PCR using degenerate primers which have 10 been designed after an alignment of SemaH with known Semaphorins and Collapsins. The sense primer is 5'- CGGGATCCAT (H) TT (Y) TT (Y) TT (Y) AC (N) GA (R) AA-3' encoding the motif YFFFTEK specific to mouse SemaH and its putative chick homologue Collapsin 5, and the antisense 15 primer is 5'-GC GGATCCTCCCA (N) GC (R) CA (R) TA (N) GG (R) TC-3' complementary to the sequence encoding the motif DPYCAWD common to all known semaphorins and collapsins. The primers encode *Bam*III sites to facilitate cloning. The PCR reaction is performed on cDNA from human fetal 20 brain and lung (Clontech) using 5 initial cycles with low annealing temperature followed by 30 cycles with high annealing temperature. The primers amplify a region of approximately 900 bp. The obtained fragments are gel purified and cloned into the *Bam*HI site of a 25 pQE30 vector (Qiagen) fused to the vector encoded N-terminal His tag to generate the pQE30/Human-His construct. This facilitates expression and purification of the corresponding peptide in M15 bacteria using the QiaExpressionist system (Qiagen). 30 Sequence analysis is performed using software of the Genetics Computer Group (Wisconsin, USA) to look for

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evolutionary relationship between the obtained fragments and mouse SemaH. Expression of the Human homologue is examined by Northern hybridization to RNA from human Tumor Cell lines.

5 A lambda cDNA library is made from mRNA isolated from selected human tumor cell lines with the CAP-cDNA synthesis kit and ligated into UNI-ZAP XR vectors with the ZAP-cDNA Gigapack III Gold Cloning Kit (STRATAGENE). Commercial cDNA libraries from human
10 fetal brain and lung are obtained (Clonetech or Invitrogen). The different libraries are screened with mouse semaH cDNA and human cDNA fragments isolated as described above. Obtained cDNA clones are subjected to sequence analysis to look for splice-variants, and
15 the expression of these are evaluated comparatively in tumor and normal tissue by RT-PCR and northern hybridization.

The open reading frame of the human homologue is cloned into expression vectors for the purpose of
20 transfection and protein expression analogous to mouse semaH above.

Example 4 - Expression of SemaH peptides, proteins and fusion proteins

A. E. Coli

25 M15 bacteria are transformed with the pQE30/Human-His construct and the pQE30/Mouse-His construct, and transformants selected for ampicillin resistance. Selected transformants are grown in LB medium, and the expression of the fusion proteins
30 induced by IPTG and purified from total protein lysate through the use of a Ni²⁺ resin, following the guidelines of the QIAExpressionist system (Qiagen).

B. *Pichia Pastoris*

Mouse SemaH-TMH is expressed in the yeast *Pichia Pastoris* using the strains KM71, GS115 and X33 and guidelines of the EasySelect kit (Invitrogen). The 5 pPICZ \square B/SemaH-TMH construct is linearized using *SacI* restriction enzyme, and transformed into the yeast cells by electroporation. Cells are plated on agar plates containing yeast extract, peptone, dextrose, sorbitol, and from 100 μ g/ml-2000 μ g/ml Zeocin. Highly 10 resistant clones are selected, cultured and induced with 1% v/v methanol, and tested for secretion of the SemaH-TMH to the media. Detection of the fusion protein is done by western hybridization using anti-mye antibodies (Dept. of Cell Cycle and Cancer, the Danish 15 Cancer Society), anti-his antibodies (Qiagen), and SemaH specific antibodies. SemaH is purified using a Ni $^{2+}$ resin according to the instructions of the QIAexpressionist system (Qiagen), combined with affinity purification using anti-mye antibodies coupled 20 to CNBr activated sepharose.

Example 5 - Production of antibodies to mouse and human SemaH

The full-length SemaH protein or SemaH specific peptides are injected into rabbits and rats 25 for the production of polyclonal antibodies as well as in mice for the production of monoclonal antibodies. Hybridomas are produced according to methods well known in the art, for example, through fusion of mouse spleen lymphocytes with NS-2 cells. Cells are cultured in 30 conditioned media, and screened for production of immunoglobulins binding SemaH protein in ELISA assays and, when immobilized on membranes, by western

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hybridization. The epitope specificity of selected hybridomas is determined by ELISA on a display of 20 amino acid peptides spanning the antigen with 5 amino acid overlap, each peptide is immobilized in a 5 streptavidin coated ELISA well through biotinylation linkage.

Example 6 - Assay System for Metastatic Conversion

A cell line system developed by Cheryl J. Aslakson and Fred R. Miller at the Michigan Cancer 10 Foundation (Aslakson, G.J. and Miller, F.R., "Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor," Cancer Research, 52, 1399-1405, 1992) is used. 67NR is a non-invasive cell line which 15 is resistant to neomycin. 168FARN is a neomycin and 2,6-diaminopurine resistant cell line which is invasive, metastasizing to the lymph nodes, but not to the lungs. A non-metastatic cell line obtained in our lab, CSML-O, has been made neomycin resistant. Cells 20 are transfected with the pcDNA3.1/semaH/zeo^R and pcDNA3.1/semaH-TMH/zeo^R constructs. Stable transfectants are injected into Balb/c (67NR and 168FARN) and A/sn (CSML-O) mice intravenously, subcutaneously, or in the mammary pad of female mice. 25 Non-transfected cells will be injected in parallel as controls.

Following the guidelines of Aslakson et al., to track the path and extent of metastasis, we isolate cells from blood, lymph nodes, liver and lungs. 30 Injection of 1 x 10⁵ cells subcutaneously and in the mammary is followed by the sacrifice of groups of 5 mice at different time points thereafter. Upon

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removal, blood is heparinized. Lungs and lymph nodes are digested by Collagenase IV and Elastase (Sigma Chemical Company, ICN Biochemicals) followed by dispersion in a blender (Tekmar Company). The liver is 5 treated with Collagenase type I (Sigma) and Hyaluronidase (Sigma) followed by dispersion in the blender. Aliquots of tissue sample are plated in DME-10 media containing 10% fetal calf serum and containing combinations of the antibiotics neomycin (Gibco BRL), 10 2,6-diaminopurine (Sigma) and Zeocin (Invitrogen) appropriate to the cell line and plasmid. After 10-14 days the number of colonies per organ are calculated.

Differences between the transfected and non-transfected cells in the ability to survive selective 15 events such as invasion, diffusion in the blood or integration in the lungs/liver indicates metastatic conversion.

A. Mouse

Following injections in the *subcutis* and in 20 the mammary pad of female mice with 1×10^5 cells from non-metastatic, metastatic or semaH transfected cell lines, groups of 5 mice are sacrificed at different time points. Serum is collected from each sample, and the levels of SemaH protein are examined by ELISA using 25 SemaH monoclonal antibodies. SemaH protein is quantified by calorimetric means or by radioactively labeled secondary antibodies followed by scintillation of samples. The amount of SemaH protein is correlated with parameters such as the size of the primary tumors, 30 the pathological stage of tumor sections, immunostaining of such sections with SemaH antibodies, the presence of visible metastasis, and the presence of

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clonogenic tumor cells in the blood, lymph nodes, lungs and livers obtained through the exploitation of the antibiotic resistance of the cells injected analogous to above.

5 B. Humans

Tissue specimens from patients with diagnosed and staged cancer are obtained. Immunohistochemistry is performed on tissue sections using the anti-SemaH monoclonal antibodies. Metastatic conversion is
10 detected by antibody binding.

As will be appreciated by one of skill in the art, the methods and compositions disclosed herein are used for making and identifying diagnostic probes, therapeutic drugs and kits for same.

15 All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although
20 the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes
25 and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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What is claimed is:

1. An isolated, recombinant or synthetic DNA molecule comprising a DNA sequence encoding a semaphorin-H polypeptide, wherein the DNA sequence is
5 selected from:

(a) the DNA sequence of SEQ ID NO:1;

(b) the DNA sequence of SEQ ID NO: 2;

(c) the DNA sequence contained in the insert of clone ORF (EMBL No. 80941);

10 (d) fragments of the DNA sequences of (a-c);

(e) DNA sequences which hybridize under stringent conditions to the DNA sequences of (a-c) and which code for a polypeptide displaying Semaphorin-H
15 activity; and

(f) DNA sequences which encode a polypeptide encoded by a DNA sequence of (a-e).

2. The isolated, recombinant or synthetic DNA molecule according to claim 1, operatively linked
20 to an expression control sequence.

3. Host cells transformed with a DNA molecule according to claim 1 or claim 2.

4. A method for producing a polypeptide comprising the step of culturing a host cell according
25 to claim 3.

5. Host cells expressing the polypeptide encoded by the DNA of claim 1.

6. The polypeptide produced by the method according to claim 4.

5 7. An isolated, recombinant or synthetic polypeptide selected from the group consisting of:

(a) the polypeptide having the amino acid sequence set forth in SEQ ID NO: 3;

10 (b) the polypeptide having the amino acid sequence set forth in SEQ ID NO: 4;

(c) the polypeptide encoded by the DNA sequence contained in the insert of clone SemaH (EMBL NO.93947);

15 (d) fragments of the foregoing polypeptides having at least 5 amino acids;

(e) a polypeptide encoded by a DNA sequence that hybridizes under stringent conditions and that is at least 80% complementary to a DNA sequence according to claim 1; and

20 (f) derivatives of any of the foregoing polypeptides.

8. A fusion protein comprising a polypeptide according to claim 6 or claim 7.

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9. A multimeric protein comprising a polypeptide according to claim 6 or claim 7.

10. A pharmaceutical composition comprising a polypeptide according to claim 6 or claim 7; a fusion 5 protein according to claim 8; or a multimeric protein according to claim 9.

11. An antibody that specifically binds to a polypeptide according to claim 6 or claim 7.

12. The antibody according to claim 11, 10 wherein the antibody is polyclonal.

13. The antibody according to claim 11, wherein the antibody is monoclonal.

14. An antibody according to claim 11 or 13, wherein said antibody inhibits semaphorin activity.

15 15. The antibody according to claim 11, 13 or 14 wherein said antibody is labeled.

16. The antibody according to claim 15 wherein said label is selected from the group consisting of enzymes, fluorochromes, radioisotopes, 20 and luminescers.

17. A hybridoma cell line that produces an antibody according to claim 13.

18. A method for determining the metastatic 25 potential of a cell, comprising the step of detecting

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in a biological sample the expression of a polypeptide according to claim 7.

19. The method according to claim 18,
comprising the step of contacting a biological sample
5 with an antibody according to any one of claims 11-16
and detecting the formation of an antibody-antigen
complex.

20. The method according to claim 19,
10 wherein the step of detection is by enzyme reaction,
fluorescence, luminescence emission, or radioactivity
measurements.

21. A method for detecting the metastatic
potential of a cell, comprising the step of assaying a
15 biological sample for the presence of RNA encoded by a
DNA sequence according to claim 1.

22. The method according to claim 21,
wherein the assay is RT-PCR or Northern assay.

23. A diagnostic kit comprising an antibody
20 according to any one of claims 11-16, or a protein
according to claim 6 or claim 7.

24. A diagnostic kit comprising a primer
derived from a DNA sequence of claim 1.

25. A method for inhibiting metastasis,
comprising the step of inhibiting the biological
activity of a polypeptide according to claim 6 or claim
7.

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26. The method according to claim 25,
comprising the step of contacting the cells with a
molecule selected from the group consisting of: an
antibody according to any one of claims 11-16; a small
5 molecule inhibitor; and a semaphorin-H ligand or
fragment thereof.

27. A method for inhibiting metastasis of
cells expressing a DNA sequence according to claim 1,
comprising the step of preventing the expression of
10 said DNA sequence.

28. The method according to claim 27,
wherein the method utilizes an antisense nucleic acid
derived from a DNA sequence according to claim 1.

29. A composition comprising an antibody
15 according to claim 11-16 and a pharmaceutically
acceptable carrier.

30. An anti-sense oligonucleotide derived
from a DNA sequence according to claim 1.

20 31. A pharmaceutical composition comprising
the antisense oligonucleotide according to claim 30.

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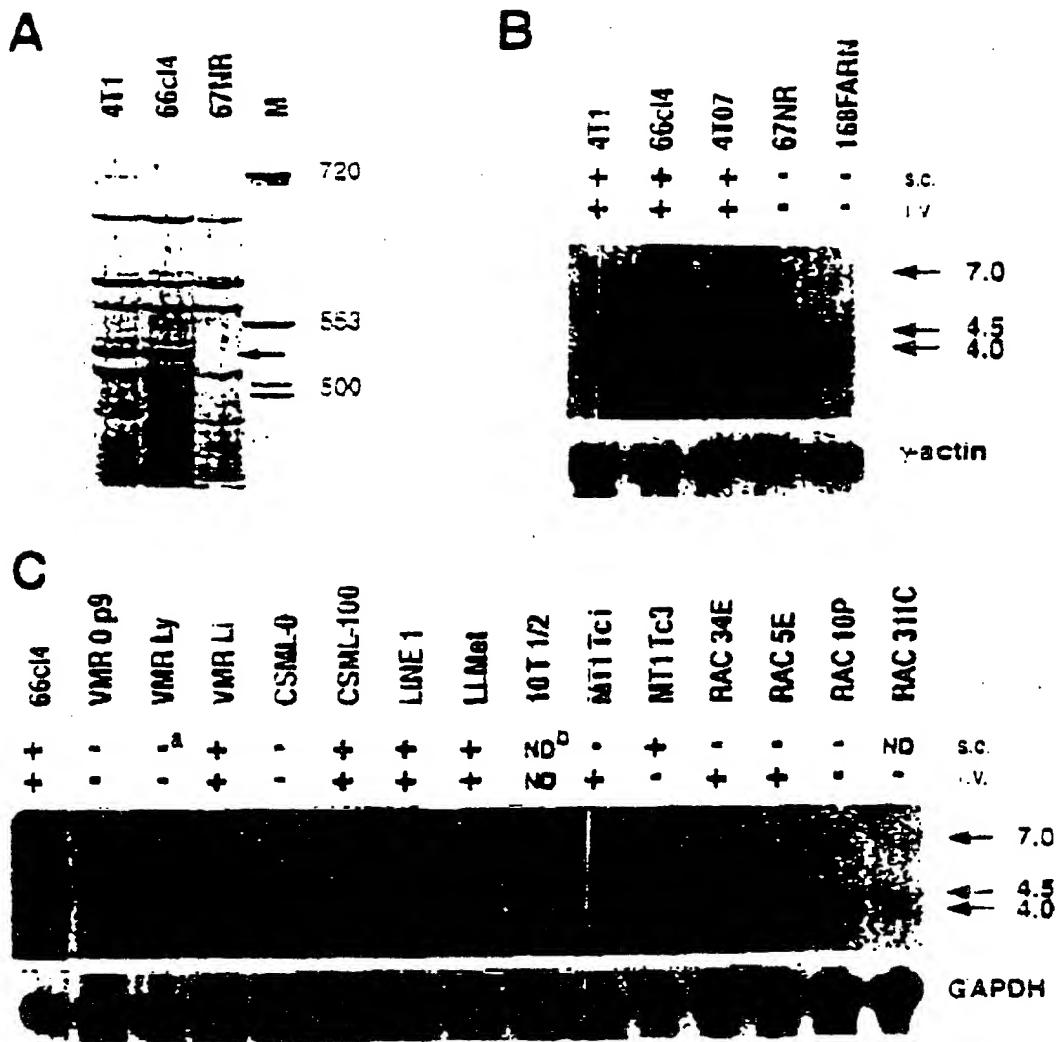


FIGURE 1

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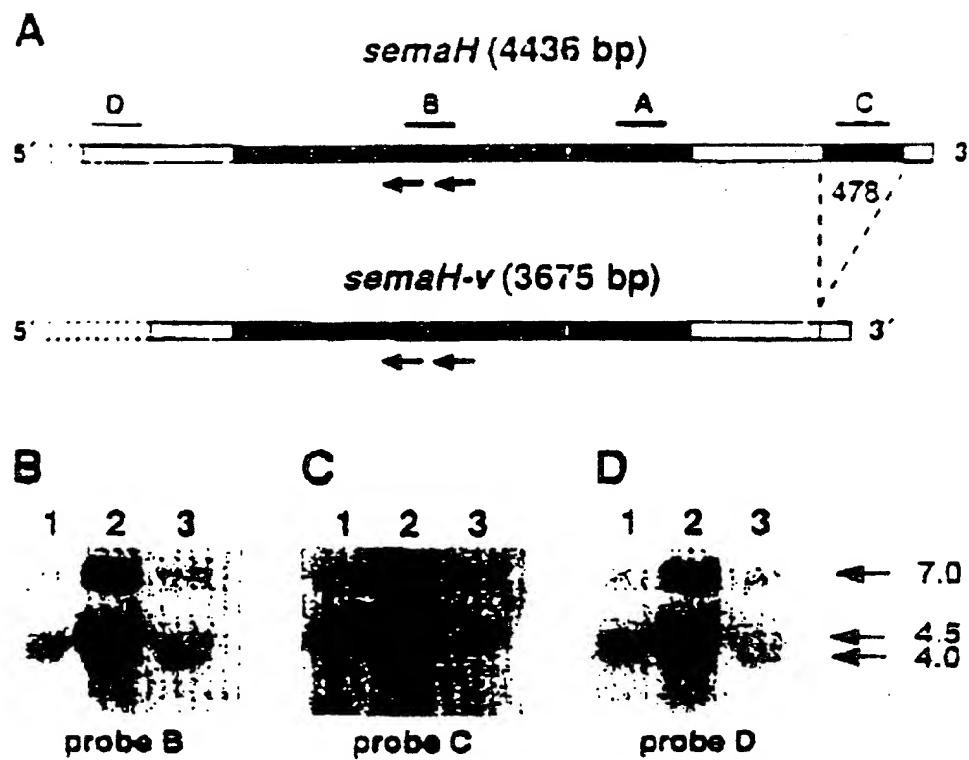


FIGURE 2

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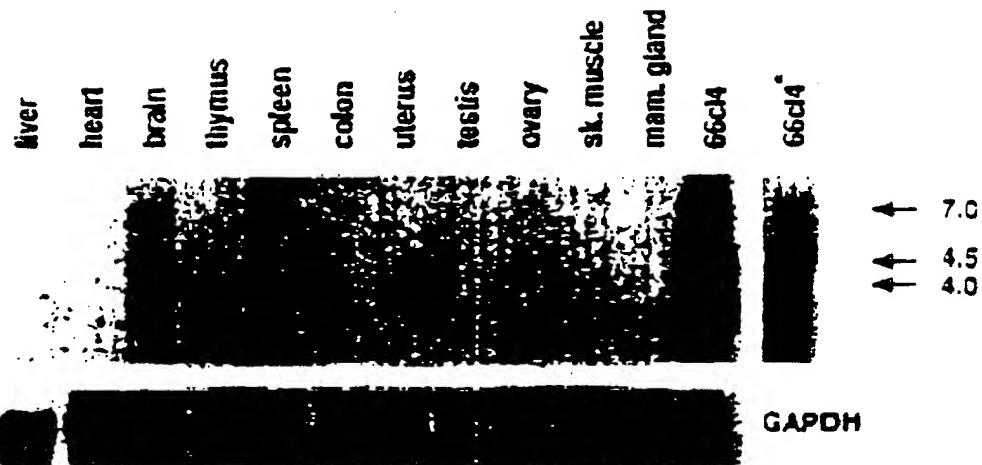
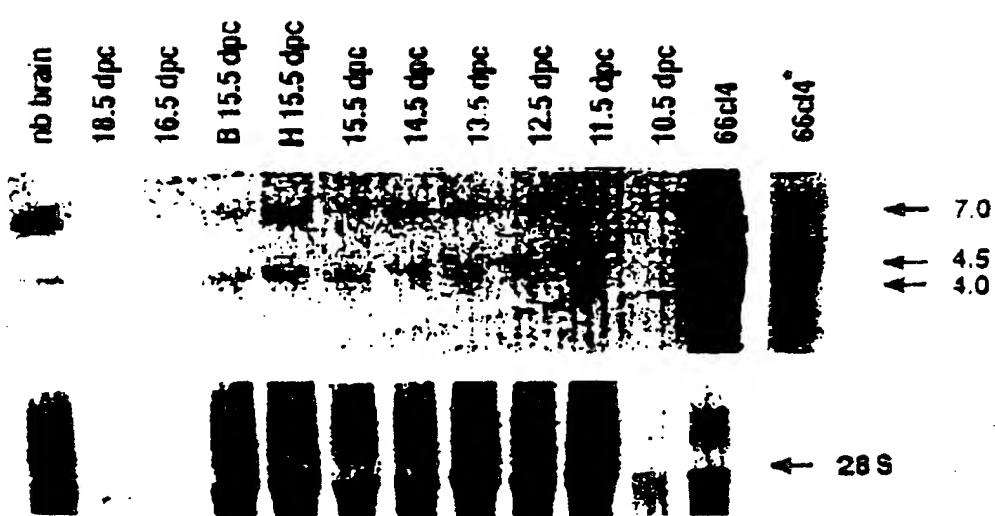
A**B**

FIGURE 3

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M-Semal	W K A P L G F I D C [S T W] D E Y Q E R R F V E C R E L V Y S I S C R V A S D - C Y R E [S Y P S	19
C-Coll5	- - - - -	3
B-Semal	W W G L A N S S S T [S T Y] D E Y Q E R R F V E C R E L V Y S I S C R V A S D - C Y R E [S Y P S	41
D-Semal	W W G G K L Y Y R - T F E - M Y D D D D D D Y V V G A H C A Y F R V E L Q N I S - - - - -	43
G-Semal	W G N E S S K D - R Y K L E X D E N S I C Y V G A R C Y T H I S M R D L T E P T E Q R E Y S	43
*		
M-Semal	T A V K V E E C I K K G K D A - M E C A N Y T R V E E Y N R - C E C L T C A M G A P D E C A F :	77
C-Coll5	- - - - -	3
B-Semal	S T T E R D E C K X A G K D X L K E C A M P T E V E X A Y N Q - C E L Y A C G T G A P E P I C T Y I	77
D-Semal	- - - - - S N C M R D A I S - L I D C K X M R V R V Q S E D Q G D R L Y V C G T M A E N P X - D Y Y	71
G-Semal	S G A E E R L C T Y K G S - Z D D C Q M Y T R V L A R I D C - C R V L C C O N H A Y K P L - - C X	73
#		
M-Semal	R V G E S S E Z P L F E L S S R E E R C G R C F P D P E S S T V S T L T G N - - - - - Z L P	143
C-Coll5	- - - - -	3
B-Semal	E I C E S P F D W I F E L S S T Y E M G R C R S P Y P D P E L L A S L L I D G - - - - - Z E Y	149
D-Semal	I Y A M L T H L P R S S T Y V T G V G L - G I A E C P Y P D P L D S T A I Y V E N G M P O G L P G L Y	130
G-Semal	E Y A - - - - - I E D G D Y V V Z E Y T E S R G L C P P D P D E S T A I Y S E Q - - - - - Q L Y	134
*		
M-Semal	A C G L T S [S T W] D S A T P N S - - - - - N G K E G E I S T Q E D D E R L E K - E P E F V G E T X X P D S	128
C-Coll5	- - - - -	3
B-Semal	S O T A A D P T M G R D P A I P T S - - - - - C G E H E P C I T Q E D S R W L H - D P K F I S A H L I S S	188
D-Semal	S O T A A D P T K A D T V C I P T D L Y N T S A X A L T C Y F R E T E T D S K E W L D K P M F V G S	180
G-Semal	S A T V A D P S T D P L A T Y R S P L - - - - - E T E S D L E Q L H A P K P V N T	171
*		
M-Semal	E D R D D S E X M Y Y P P T E X C A I D E A E H A S T T I S Y G R E C V H D M G G Q H I L V H K Y S T	238
C-Coll5	- - - - -	16
B-Semal	D P E D D D E V Y Y P P T E X C A I D E A E H A S T T I S Y G R E C V H D M G G Q H I L V H K Y S T	238
D-Semal	F D I C E Y - Y Y P P T E X A T V Y T H C G A V Y V S R I A B V C E K A D U V G G C H E L I M A E H M A T	229
G-Semal	H X Y V D P - I Y Y P P T E X A T V A V Y I M C Q A I T S R V A R V C X E D K G G P E Q F G D R E T S	239
*		
M-Semal	P L I X A Z L V C S T P G M M G I D T X F D Z D H D V F G L - - - - - P T R D P K M P V I P G L F M T P S	245
C-Coll5	P L I X A Z L V C S V P G M M G I D T X F D Z D H D V F G L - - - - - Q T R D P K M P V I P G L F M T P S	93
B-Semal	P L I X A Z L V C S V P G M M G I D T X F D Z D H D V F G L - - - - - M F X D P K M P V V V Y G V F T P S	271
D-Semal	Y L I X A R L H C S Y D G - - - - - E P P P T F H E Z I S B V T Q D P S D X - - - - - S X P A T P T T S	238
G-Semal	P L I X S R P H C S V P G - - - - - C P P P T F H E Z I S B V T Q D P S D X - - - - - S X P A T P T T S	238
*		
M-Semal	W T P R G S A V C V M I P J D V I R R V Y P L G Y T A E R D G P Y T C K V P T O G - - - - - R V P T P R P G T C	316
C-Coll5	W T P R G Y A C C V I X A I V A A P M G Y T A E R D G P Y T C K V P T O G - - - - - R V P T P R P G T C	143
B-Semal	W T P R G S A V C V M I P J D V I R R V Y P L G Y T A E R D G P Y T C K V P T O G - - - - - R V P T P R P G T C	316
D-Semal	M G L I G S A V C I S E H E I G A A P M G Y T A E R D G P Y T C K V P T O G - - - - - R V P T P R P G T C	321
G-Semal	M S I G G S A V C A P S E I S P D G P Y T C K V P T O G - - - - - R V P T P R P G G C	318
*		
M-Semal	A S E V V N G G E K Y G T H Q X P D D A I A P A X A M S T L E Y Q D I E P V E S K P D I L V K T D G S Y N	314
C-Coll5	A S E V V N G G L Y T T P D D T P D D A I A P A X A M S T L E Y Q D I E P V E S K P D I L V K T D G S Y N	293
B-Semal	P S E T P G Q - P D D T P D D T P D D T P A R S E P A N Y I P V P M H E R P I V A I X T D V Y Y Q	383
D-Semal	V - - - - - E D T S M L P D T V L M P T R S E P L M D K A V N E S E H V I Y T A R D L - - - - - V	361
G-Semal	V - - - - - E D S I T L P D V S V N V T A S E P L M D K A V N E S E H V I Y T A R D L - - - - - V	360
*		
M-Semal	L A Q L L V V D - - - X V E A S D G Q T D V L F I G T D P G I V L L K V I P I Y M Q S T E H N M E E V I L	622
C-Coll5	L A Q L L V V D - - - X V E A S D G Q T D V L F I G T D P G I V L L K V I P I Y M Q S T E H N M E E V I L	239
B-Semal	I T Q I V V T D - - - X V D A E D G Q Y D V M P C G T D P G I V L L K V I P I Y M Q S T E H N M E E V I L	439
D-Semal	Y T Q L V V D E I I T D I L M Q E Y T V Y V T K L G I C T Y K I V Q - - - T R N C E S L S K I - - - - - V	407
G-Semal	P T R I S A V D S Q V R T Q D S K Y P I T S D C R V I K A L H S A S P D S S D T V D S V I	610
*		
M-Semal	E S L Q I P F Z D P A P - - - T I S S K E T S S K E Q Q L Y I O S S A V A Q V Z P F E E C D M T G - S A	678
C-Coll5	E S L Q V F Z D P I P - - - T I S S K E T S S K E Q Q L Y I O S S V I A Q V Z P F E E C D M T G - S A	285
B-Semal	E S L Q V F Z D P I P - - - T I S S K E T S S K E Q Q L Y I O S S V I A Q V Z P F E E C D M T G - S A	176
D-Semal	- - C D P F P E V A - Y H E A L I Q V W R I S Q T A K S T I G E D E R T E I D L I Q D V A N T S - D E C	653
G-Semal	E P L Q V Z P P G V P V K L L T V V R K D D E K I T V V I D D D S X L A L E R C G S D R I T H	460
*		
M-Semal	C A D C C L A R D Y C A V D - - - G I S C S A T Y T G A E E Z A P F R A D V & E Q M A A Q Q C	525
C-Coll5	C A D C C L A R D Y C A V D - - - G I S C S A T Y T G A E E Z A P F R A D V & E Q M A A Q Q C	525
B-Semal	C A B C C L A R D P I C A V E - - - G I S C S A T Y T G A E E Z A P F R A D V & E Q M A A Q Q C	491
D-Semal	C F F R C V - - - R Q P Y C G W D R E A N T C I P Y - - - - - E L D L I Q D V A N T S - D E C	528
G-Semal	C B E C V S L Q D P Y C A W D N V E L A C T A V G S P D V E A G K E P F I O N Z S L Q E - X A S	528

FIGURE 4A

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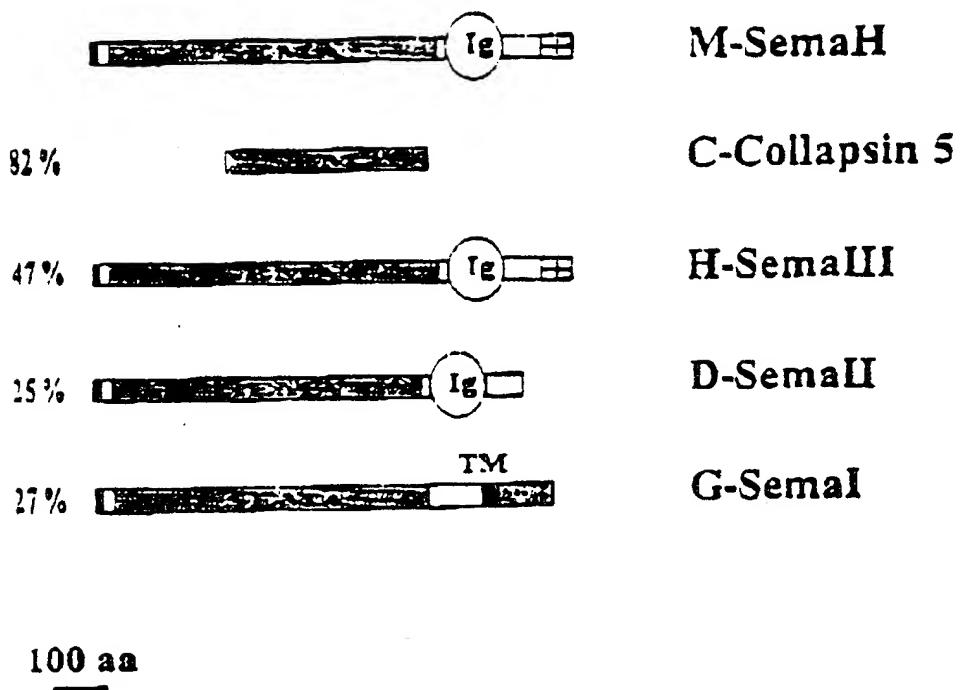


FIGURE 4B

- 1 -

SEQUENCES

SEQ ID NO. 1 SemaH DNA sequence

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5 51 TTTCTGTAA ACAGAGCGCT GACAGGCGGC ATCCCCGCTG
GGTGGATCCC

101 101 GCGCCCTGGC GCAAGTGGCA CTTCTTGCTT CTAATTATCG
AGAGGAGAGG

151 151 CGAATACGAA CTAGCTGCTC GGCAAGTCAG TGTCAGGAGG
10 CTGACTTCTG

201 201 GGAGGCTGGC GGGGAGGCTG GGGGAAGAGC TGGGGGAGGC
TGCTGCTCTG

251 251 CTTCGACTGT TTTCTCAATG AATAGCTGGC GGGGAGACTG
AAGCTAGCCA

15 301 301 CAGCCTCCTC CTTCACTCCG CGTCTGGCT GACGGCGACA
GCACCAGCCC

351 351 GGACCTGGCT CTCAAGACGC GCTCCTTGGA CGGTCTCTTG
CTCCGCGCTT

401 401 CTAACCACCG GGCCAAAGA CAGAAAGGCT TAGCGGATCC
20 AAATATTGCC

451 451 CGGCAAATGG CACTTGGGAA TGGTATTTTC TGATGACAAC
CCCTTCTGTT

501 501 TGTGACAAAG CCTGTCGCC GCCAGTTGCC CCTGGAGGGA
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- 2 -

551 AAAACTCAAT CCTGTCTTAA AGTGTGGCTG CAGGGGCCAG
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601 GCACGCACCA TGGCACCGGC CGGACACATC CTCACCTTGC
TGCTCTGGGG

5 651 TCACCTGCTG GAACTCTGGA CCCCAGGTCA CTCCGCGAAC
CCCTCCTACC

701 CCAGGCTACG CCTGTCACAT AAAGAACTTT TGGAAC TGAA
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751 ATATTTCAAA GCCCCCTTGG ATTTCTTGAT CTCCATACAA
10 TGCTGCTGGA

801 TGAGTATCAA GAACGGCTCT TTGTGGGAGG CAGAGACCTT
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851 TGAACTTGGA ACGAGTCAGT GACGGCTACA GAGAGATATA
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15 901 ACAGCAGTAA AGGTAGAAGA ATGCATAATG AAAGGAAAAG
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951 GTGTGCCAAT TATATCCGGG TTTGCATCA CTACAACAGG
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1001 TGACCTGTGC TACTGGAGCT TTTGATCCAC ACTGTGCCTT
20 CATCAGAGTC

1051 GGGCACCATT CAGAGGAACC CCTGTTTCAC CTGGAGTCAC
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1101 GAGAGGAAGG GGCAGATGTC CTTTGACCC CAACTCCTCC
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1151 CGCTAGTTGG GAATGAGCTG TTTGCTGGAC TCTACAGTGA
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1201 AGAGACTCGG CGATCTTCCG CAGCATGGGG AAGTTAGGCC
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5 1251 TGAGCATGAC GATGAGCGGC TCCTGAAAGA ACCAAAATTT
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1301 ATATGATTCC TGATAACGAA GACCGAGATG ACAACAAAAT
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1451 TGAACAAGTG GAGCACTTTC CTTAAAGCGC GGCTGGTTG
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15 1501 GGAATGAATG GAATCGACAC ATACTTTGAC GAACTAGAGG
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1701 ATACCACTGG TCACTATATG AAGGAAAAGT CCCCTACCCA
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- 4 -

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5 1851 CATAAAACCT GTTCATAAAA AACCAATACT GGTAAAAACA
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1901 ACAACCTGAG GCAACTTGCC GTGGATCGGG TGGAAGCGGA
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1951 TATGACGTCT TATTTATTGG GACAGACACA GGAATTGTGC
10 TGAAAGTAAT

2001 CACAATTAC AACCAAGAAA CAGAGTGGAT GGAGGAAGTC
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2051 AACTCAAAT ATTCAAGGAT CCAGCCCCTA TCATTCTAT
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15 2101 TCAAAGAGAC AACAGCTTA CATTGGATCA GCCTCTGCTG
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2151 CAGATTCCAT CACTGCGACA TGTATGGCAG TGCTTGTGCT
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2201 TGGCTCGAGA CCCGTACTGT GCCTGGGATG GCATATCCTG
20 CTCCAGGTAC

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2301 GCATGGCAAC GCCGCCAAC AGTGCTTTGG ACAGCAATTT
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5 2451 TGTACAGAAG GGACGCGACG TAAGAAAAGA AGAGGTGAAG
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15 2701 CCCTTAAGCG GTATGTCTCA GGGGACAAAAA CCGTGGTACA
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2751 GCAGCTGATT GGCTACAGCA ACTTCCAGAG AGTGGAAAGAA
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2801 AGGTGTGGTG TACAGATAAG AAGAGGAAAAA AGCTTAAAAT
20 GTCTCCCTCC

2851 AAGTGGAAAGT ATGCCAACCC CCAGGAAAG AGGCTTCGCT
CTAAAGCTGA

2901 GCACTTCCGC CTGCCAGGC ACACGCTGCT CTCCTGAGGG
CGCCCTCTGC

- 6 -

2951 CGGC GGCTGA GGAACCTAGG ATGGAAACAT TTTTTAAAG
GGGGGGAAAAA

3001 AAAAAAGACT GAAAGCATGC AGTCTCTTC ATTACTCAA
AGAACTTTCT

5 3051 GTAGTACTGA GAGGCTGGGA AGGTGTTTA AGTTATTCTG
CATATCATCT

3101 GACTGTACAA ACATCTCTCT CACAGTACTA GTACTTAAAC
GAGTGTATTG

3151 CTTTAAAAGG CTTCACCTAT TTATCAACAT ATGATCGGGC
10 TGTAATTTG

3201 AGCTTGCTTT TCAGGGAAAT TATTTGCCA TTGGGATATG
TATTATAATT

3251 ATAATATCAA AGAAAAAGTT ATAGGTTTC ATAATCAAGA
AAAATGTAAA

15 3301 ACTCTTCTG GAGTTTTTC TTTTTCTTT TCCTCTTGC
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3351 TAAAATATAC AGAATATCAG ATATTGTAGA ATTATCGTGT
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3401 GTCAGTTGTT GATTTTTTT TTTAAGCCAT AAGTTGTATG
20 TAGCTGTGTT

3451 GTGGTTGCTA TAAACTGTAC AAGAAATCTG CAACCCATCC
ATTCTGAGGC

3501 TTGTTCTCTT CATGAAACAC TCACTAAACT ATATTAAGAA
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- 7 -

3551 TTATAAACAA GACATATCTT TTAAATTCA CTTCAGTGTG
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3601 AGATAGCCTC TCAGAAAGAT ATTTTAAAT AGTTCAAAC
CATGAAAGTA

5 3651 GAATTCTGTT AAAAACGGTC TGTGCTTCCC TCTTGTGGTA
CAAGAAGGCA

3701 TTCGATCTCT CAATGCTGTT TCCATAATTT TCCTCGAATG
ACTTGAAAAC

3751 AAAATGTAGT AGATACATCT AGTTCACTAT TCAGAAGACT
10 CAATTAAAAA

3801 ATAATTTAT ATAATAATTT AATATGGAAA TAGCATGCTT
CTGGTTAATT

3851 CATTACTTCA CAACAAACCG AAGATGTTAA CTGCATTTGA
GCTTTGAAAT

15 3901 AGAAGAAAGT ACCTTCCAG TCCTACCCCTG AGACAAGACA
CTCTGAAAGT

3951 GTGCTTACC CGCAAAGAAA TTATCACCGA TTCTTTCTC
AGAAATGAAA

4001 TCTTCCTATT TTATATTTAC TTCTGGATT CGTTTTACTA
20 CTGACATCTT

4051 TAGAGAATCA TTATCTTGT CTTATGAATA TAATCAAAAT
TTATATTTTT

4101 CAGAATGTTT ATTTCTTGAG ATTTTCTTC TCTGTATTTT
AAAGAGCCAG

- 8 -

4151 CAGCTTCGGT TCTAAAATAT GATTTTATG AGGCTTTCA
GTTCGGTAAG

4201 AGTAACGCAA GTGGATTGTA ATAAGAACAA TTATGAATAC
GTATTCAGAC

5 4251 TTAAATTAA CTATTATGTC AGCTATCTCA GTATGCTATA
TAATTAAGAA

4301 TATAAAGTGT TGAGAACTTC TAATGGATT CTTTTCTGT
CTAAAATTAG

10 4351 GTACTACATA AATATTCCAC AATTTCAAA TTTTAAAATT
TTGGAAATTT

4401 TAAAGTCCAG AATTCTAAA ACATATAACT AGTATGAATA
AAGAGAAATGT

4451 TGACATCCTC

SEQ ID NO: 2 SemaHv DNA sequence

15 1 AATTGGCAC GAGTTCTAG AAACGCGCGC GGGCTCAACC CTGCCTGAAC

51 TTTCTGTAA ACAGAGCGCT GACAGGCGGC ATCCCCGCTG GGTGGATCCC

101 GCGCCCTGGC GCAAGTGGCA CTTCTTGCTT CTAATTATCG AGAGGAGAGG

151 CGAATACGAA CTAGCTGCTC GGCAAGTCAG TGTCAGGAGG CTGACTTCTG

201 GGAGGCTGGC GGGGAGGCTG GGGGAAGAGC TGGGGAGGC TGCTGCTCTG

20 251 CTTCGACTGT TTTCTCAATG AATAGCTGGC GGGGAGACTG AAGCTAGCCA

301 CAGCCTCCTC CTTCACTCCG CGTCTGGCT GACGGCGACA GCACCAGCCC

- 9 -

351 GGACCTGGCT CTCAAGACGC GCTCCTTGGA CGGTCTCTTG CTCCGCGCTT
401 CTAACCACCG GGCCCAAAGA CAGAAAGGCT TAGCGGATCC AAATATTGCC
451 CGGCAAATGG CACTTGGGAA TGGTATTTTC TGATGACAAC CCCTTCTGTT
501 TGTGACAAAG CCTGTCGCC CCGAGTTGCC CCTGGAGGGA AGTACTAAGT
5 551 AAAACTCAAT CCTGTCTTAA AGTGTGGCTG CAGGGGCCAG AGGAGAGCCA
601 GCACGCACCA TGGCACCGGC CGGACACATC CTCACGTTGC TGCTCTGGGG
651 TCACCTGCTG GAACTCTGGA CCCCAGGTCA CTCGGCGAAC CCCTCCTACG
701 CCAGGCTACC GCTGTCACAT AAAGAACTTT TCGAACTGAA TGGACTTCAA
751 TATTTCAAAG CCCCCCTTGG ATTTCTTGAT CTCCATACAA TGCTGCTGGA
10 801 TGAGTATCAA GAACGGCTCT TTGTGGGAGG CAGAGACCTT GTCTATTCCC
851 TGAACTTGGA ACGAGTGAGT GACGGCTACA GAGAGATATA CTGGCCGAGC
901 ACAGCAGTAA AGGTAGAAGA ATGCATAATG AAAGGAAAAG ACGCAAATGA
951 GTGTGCCAAT TATATCCGGG TTTGCATCA CTACAACAGG ACACACCTTC
1001 TGACCTGTGC TACTGGTGCT TTTGATCCAC ACTGTGCCTT
15 CATCAGAGTC
1051 GGGCACCATT CAGAGGAACC CCTGTTTCAC CTGGAGTCAC
ACAGATCTGA
1101 GAGAGGAAGG GGCAGATGTC CTTTGACCC CAACTCCTCC
TTTGTGTCCA

- 10 -

1151 CGCTAGTTGG GAATGAGCTG TTTGCTGGAC TCTACAGTGA
CTATTGGGGC

1201 AGAGACTCGG CGATCTTCCG CAGCATGGGG AAGTTAGGCC
ATATTCGCAC

5 1251 TGAGCATGAC GATGAGCGGC TCCTGAAAGA ACCAAAATTT
GTAGGTTCAT

1301 ATATGATTCC TGATAACGAA GACCGAGATG ACAACAAAAT
GTACTTTTC

1351 TTTACTGAGA AGGCGCTGGA GGCGGAGAAC AACGCCAC
10 CGATCTTACA

1401 CCCGAGTGGG CGGCTGTGCG TGAATGACAT GGGAGGACAG
AGAATCCTGG

1451 TGAACAAGTG GAGCACTTTC CTTAAAGCGC GGCTGGTTG
CTCAGTGCCG

15 1501 GGAATGAATG GAATCGACAC ATACTTGAC GAACTAGAGG
ATGTGTTTT

1551 ACTGCCGACC AGAGATCCTA AGAATCCAGT GATATTTGGA
CTGTTAATA

1601 CTACCAGCAA TATATTTAGA GGCCATGCTG TATGTGTGTA
20 TCACATGTCA

1651 AGTATCCGGG AAGCCTTAA TGGCCCATAT GCTCATAAAG
AAGGCCCTGA

1701 ATACCACTGG TCACTATATG AAGGAAAAGT CCCCTACCCA
AGGCCTCGTT

- 11 -

1751 CCTGTGCCAG CAAAGTAAAC GGAGGCAAGT ATGGAACCAA
CCAAAGATTA

1801 CCCGATGACG CCATCCGGTT CGCAAGGATG CATCCTCTAA
TGTATCAGCC

5 1851 CATAAAACCT GTTCATAAAA AACCAATACT GGTAAAAACA
GATGGAAAAT

1901 ACAACCTGAG GCAACTTGCC GTGGATCGGG TGGAAGCGGA
GGATGGCCAG

1951 TATGACGTCT TATTATTGG CACAGACACA GGAATTGTGC
10 TGCTGAAAGT

2001 AATCACAATT TACAACCAAG AAACAGAGTG GATGGAGGAA
GTCATTCTAG

2051 AGGAACCTCA AATATTCAAG GATCCAGCCC CTATCATTTC
TATGGAAATT

15 2101 TCTTCAAAGA GACAACAGCT TTACATTGGA TCAGCCTCTG
CTGTGGCACA

2151 AGTCAGATTC CATCACTGCG ACATGTATGG CAGTGCTTGT
GCTGACTGCT

2201 GCCTGGCTCG AGACCCGTAC TGTGCCTGGG ATGGCATATC
20 CTGCTCCAGG

2251 TACTACCCAA CAGGTGCACA CGAAAAGAGG AGGTTCCGCA
GGCAGGACGT

2301 TCGGCATGGC AACGCCGCC AACAGTGCTT TGGACAGCAA
TTTGTGGAG

- 12 -

2351 ACGCGTTGGA CAGGACTGAA GAGAGGCTGG CTTATGGCAT
AGAGAGCAAC

2401 AGTACTCTGT TGGAATGCAC CCCGCTATCA CTACAAGCAA
AAGTCATCTG

5 2451 GTTTCTACAG AAGGGACGCG ACGTAAGAAA AGAAGAGGTG
AAGACGGATG

2501 ACAGAGTTGT CAAGATGGAC TTGGGCTTGC TCTTCCTCAG
AGTACGCAAG

2551 TCAGATGCAG GGACCTATT TTGCCAGACA GTAGAACACA
10 ATTTTGTCCA

2601 TACTGTGCGT AAAATCACCT TGGAGGTGGT CGAAGAGCAT
AAAGTGGAGG

2651 GCATGTTCA TAAGGACCAT GAAGAGGAAA GACATCACAA
GATGCCCTGC

15 2701 CCTCCCTTAA GCGGTATGTC TCAGGGGACA AAACCGTGGT
ACAAGGAATT

2751 CTTGCAGCTG ATTGGCTACA GCAGCAAGTT CCAGAGAGTG
GAAGAATACT

2801 GCGAAAAGGT GTGGGTACA GATAAGAAGA GGAAAAAGCT
20 TAAAATGTCT

2851 CCCTCCAAGT GGAAGTATGC CAACCCCCAG GAAAAGAGGC
TTCGCTCTAA

2901 AGCTGAGCAC TTCCGCCTGC CCAGGCACAC GCTGCTCTCC
TGAGGGCGCC

- 13 -

2951 CTCTGCCGGC GGCTGAGGAA CCTAGGATGG AAACATTTT
TTAAAGGGGG

3001 GGAAAAAAA AAGACTGAAA GCATGCAGTC TCTTCATTA
CTTCAAAGAA

5 3051 CTTTCTGTAG TACTGAGAGG CTGGGAAGGT GTTTAAGTT
ATTCTGCATA

3101 TCATCTGACT GTACAAACAT CTCTCTCACA GTACTAGTAC
TTAAACGAGT

3151 GTATTGCTTT AAAAGGCTTC ACCTATTTAT CAACATATGA
10 TCGGGCTGTA

3201 ATTTTGAGCT TGCTTTTCAG GGAAATTATT TTGCCATTGG
GATATGTATT

3251 ATAATTATAA TATTAAAGAA AAAGTTATAG GTTTCATAA
TCAAGAAAAA

15 3301 TGTAAAACTC TTTCTGGAGT TTTTCTTTT TTCTTTCCCT
CTTGCAGCC

3351 ATAAGGTAAA ATATACAGAA TATCAGATAT TGTAGAATTA
TCGTGTGAGC

3401 TTTCTGGTCA GTTGTGATT TTTTTTTTA AGCCATAAGT
20 TGTATGTAGC

3451 TGTGTTGTGG TTGCTATAAA CTGTACAAGA AATCTGCAAC
CCATCCATTC

3501 TGAGGCTTGT TCTCTTCATG AAACACTCAC TAAACTATAT
TAAGAATATT

- 14 -

3551 TCTGTTTAT AAACAAGACA TATCTTTAA ATTCACTTC
AGTGTGGAAG

3601 GATGGAAGAT AGCCTCTCAG AAAGATATT TTAAATAGTT
TCAAACCATG

5 3651 AAAGTAGAAT TCTGTTAAA ACGGTCTGTG CTTCCCTCTT
GTGGTACAAG

3701 AAGGCATTG ATCTCTCAAT GCTGTTCCA TAATTTCTT
CGAATGACTT

3751 GAAAACAAAA TGTAGTAGAT ACATCTAGTT CACTATTAG
10 AAGACTCAAT

3801 TAAAAAATAA TTTTATATA ATTAAGAATA TAAAGTGTG
AGAACTTCTA

3851 ATGGATTCT TTTCTGTCT AAAATTAGGT ACTACATAAA
TATTCCACAA

15 3901 TTTCAAATT TTAAAATT GGAAATTTA AAGTCCAGAA
TTTCTAAAAC

3951 ATATAACTAG TATGAATAAA GAGAATGTTG ACATCCTC

SEQ ID NO: 3 Sema-H amino acid sequence

MAPAGHILLLLWGHLELWTPGHSANPSYPRLRLSHKELLELN
20 RTSIFQSPLGFSDLHTMLLDEYQERLFVGGRDLVYSLNLERVSDGYREIYWPSTAVKV
EECIMKGKDANECAKYIRVLHHYNRTHLLTCATGAFDPHCAFIRVGHHSEEPLFHLES
25 HRSERGRGRCPFDPNSSFVSTLVGNELFAGLYSDYWGRDSAIFRSMGKLGHIRTEHDD

- 15 -

ERLLKEPKFVGSSYMI PDNEDRDDNMYFFFTEKALEAENNAHTIYTRVGRLCVNDMGG
5
QRILVWKSTFLKARLVCSPGMNGIDTYFDELEDVLLPTRDPKNPVI FGLFNTTSN
10
IFRGHAVCVYHMSSI REAFNGPYAHKEGPEYHWSLYEGKVPYPRPGSCASKVNGGKYG
15
TTKDYPDDAIRFARIDPLMYQPIKPVHKKPILVKTDGKYNLRQLAVDRVEAEDGQYDV
20
LFIGTDTGIVLKVITIYNQETEWMEEVILEELQIFKDPAPIISMEISSKRQQLYIGS
ASAVAQVRFHCDMYGSACADCCLARDPYCAWDGISCSCSRYYPTGAHAKRRFRRQDVRH
25
GNAAQQCFGQQFVGDA LDRTEERLAYGIESNSTLLECTPRSLQAKVIWFYQKGRDVRK
EEVKTDDRVVKMDLGLLFLRVRKSDAGTYFCQTVEHNFVHTVRKITLEVVEEHKVEGM
30
FHKDHEERHHKMPCPPLSGMSQGTKPWYKEFLQLIGYSNFQRVEEYCEKVWCTDKK
RKKLKMSPSKWKYANPQEKRRLRSKAHFRLPRHTLLS
20 SEQ ID NO: 4 SemaHv amino acid sequence
MAPAGHILTLLWGHLLWTPGHSANPSYARLPLSHKELFELN
GLQYFKAPLGFLDLHTMLLDEYQERLFVGGRDLVYSLNLERVSDGYREIYWPSTAVKV
25 EECIMKGKDANE CANYIRVLHHYNRTHLLTCATGAFDPHCAFIRVGHHSEEPLFHLES
HRSERGRGRCPFDPNSSFVSTLVGNELFAGLYSDYWGRDSAIFRSMGKLGHIRTEHDD
30 ERLLKEPKFVGSSYMI PDNEDRDDNMYFFFTEKALEAENNAHTI LHPGRLCVNDMGG
QRILVWKSTFLKARLVCSPGMNGIDTYFDELEDVLLPTRDPKNPVI FGLFNTTSN
IFRGHAVCVYHMSSI REAFNGPYAHKEGPEYHWSLYEGKVPYPRPGSCASKVNGGKYG

- 16 -

TNQRLPDDAIRFARMHPLMYQPIKPVHKKPILVKTDGKYNLRQLAVDRVEAEDGQYDV
LFIGTDTGIVLLKVITIYNQETEWMEEVILEELQIFKDPAPIISMEISSKRQQLYIGS
5
ASAVAQVRFHHCDMYGSACADCCLARDPYCAWDGISCSCSRYYPTGAHEKRRFRRQDVRH
GNAAQQCFGQQFVGDALDRTEERLAYGIESNSTLLECTPLSLQAKVIWFLQKGRDVRK
10 EEVKTDDRVVKMDLGLLFLRVRKSDAGTYFCQTVEHNFVHTVRKITLEVVEEHKVEGM
FHKDHEERHHKMPCPPLSGMSQGTKPWYKEFLQLIGYSSKFQRVEEYCEKVWCTDKK
RKKLKMSPSWKYANPQEKRRLRSKAHFRLPRHTLLS



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(51) International Patent Classification ⁶ : C12N 15/12, 15/11, 1/15, 1/21, 5/10, 15/62, C12Q 1/68, C07K 14/47, 16/18, A61K 31/70, 38/17, G01N 33/68, 33/574 // (C12N 1/15, C12R 1:84) (C12N 1/21, C12R 1:19)		A3	(11) International Publication Number: WO 99/47671 (43) International Publication Date: 23 September 1999 (23.09.99)
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(30) Priority Data: 60/077,997 13 March 1998 (13.03.98) US		Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>	
(71)(72) Applicants and Inventors: LUKANIDIN, Eugene M. [RU/DK]; Willemoesgade 33, 1th, DK-2100 Copenhagen O. (DK). CHRISTENSEN, Clause, R., L. [DK/DK]; Gammel Kongevej 35 – 4TV, DK-1610 Copenhagen S.W. (DK).		(88) Date of publication of the international search report: 4 November 1999 (04.11.99)	
(74) Agent: KOLB, Helga; Hoffmann . Eitle, Arabellastrasse 4, D-81925 München (DE).			
<p>(54) Title: DNA SEQUENCES ENCODING SEMAPHORIN-H AND DIAGNOSIS OF METASTATIC CANCER</p> <p>(57) Abstract</p> <p>The present invention relates to DNA molecules encoding novel members of the Semaphorin family, referred to as Semaphorin-H and Semaphorin-H-v. The invention further relates to polypeptides encoded by the DNA, to antibodies that bind to the polypeptides and to compositions and methods comprising the DNA, polypeptides and antibodies. The present invention provides methods for detecting metastatic cancer and determining the metastatic potential of cells. The invention contemplates the development of cells line with varying metastatic potential for use a models for studying, diagnosing and treating metastatic disease.</p>			

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/IB 99/00495

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6	C12N15/12	C12N15/11	C12N1/15	C12N1/21	C12N5/10
	C12N15/62	C12Q1/68	C07K14/47	C07K16/18	A61K31/70
	A61K38/17	G01N33/68	G01N33/574	//(C12N1/15, C12R1:84),	

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q C07K A61K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>CHRISTENSEN C R L ET AL: "Transcription of a novel mouse semaphorin gene M-semaH, correlates with the metastatic ability of mouse tumor cell lines."</p> <p>CANCER RESEARCH.,</p> <p>vol. 58, no. 6,</p> <p>11 March 1998 (1998-03-11), pages</p> <p>1238-1244, XP002095583</p> <p>AMERICAN ASSOCIATION FOR CANCER RESEARCH,</p> <p>BALTIMORE, MD., US</p> <p>ISSN: 0008-5472</p> <p>the whole document</p> <p>---</p> <p>-/-</p>	1-31

 Further documents are listed in the continuation of box C. Patent family members are listed in annex.

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"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

8 September 1999

15/09/1999

Name and mailing address of the ISA

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Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 99/00495

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 (C12N1/21, C12R1:19)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	<p>WO 98 22504 A (FURUYAMA TATSUO ; INAGAKI SHINOBU (JP); SUMITOMO PHARMA (JP)) 28 May 1998 (1998-05-28) SEQ ID 2 of WO9822504 has 99.2% identity in a 2826 bp overlap with SEQ ID 1, and 98.3% in a 2835 bp overlap with SEQ ID 2 ---</p>	1-8
X	<p>DATABASE EMBL 'Online! accession number 015041, 1 January 1998 (1998-01-01) NAGASE ET AL: "KIAA0331" XP002114072 KIAA0331 has 89.9% identity in a 775 AA overlap with SEQ ID 3, and 87.3% identity in a 777 AA overlap with SEQ ID 4 ---</p> <p style="text-align: center;">-/-</p>	1-8

Further documents are listed in the continuation of box C.

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"&" document member of the same patent family

Date of the actual completion of the international search

Date of mailing of the international search report

8 September 1999

Name and mailing address of the ISA
European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Lejeune, R

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IB 99/00495

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL 'Online' accession number Z80941, 11 October 1996 (1996-10-11) CHRISTENSEN C C : "M. musculus mRNA for semaphorin H" XP002114073 Z80941 has 100% identity in a 2328 bp overlap with SEQ ID 1, and 98.8% identity in a 2337 bp overlap with SEQ ID 2 ----	1-8
X	DATABASE EMBL 'Online' accession number Z93947, 11 April 1997 (1997-04-11) CHRISTENSEN C: "M. musculus mRNA for semaphorin H" XP002114074 cited in the application Z93947 has 99.4% identity in a 4469 bp overlap with SEQ ID 1 ----	1-8
X	DATABASE EMBL 'Online' accession number Z93948, 11 April 1997 (1997-04-11) CHRISTENSEN C: "M. musculus mRNA for semaphorin Hv (3998 bp)" XP002114075 Z93948 has 100% identity in a 3988 bp overlap with SEQ ID 2 ----	1-8
A	HASHIMOTO ET AL: "Identification of genes differentially expressed in association with metastatic potential of K-1735 murine melanoma by messenger RNA differential display" CANCER RESEARCH, vol. 56, no. 22, 15 November 1996 (1996-11-15), pages 5266-5271, XP002095582 ISSN: 0008-5472 abstract -----	18, 21

INTERNATIONAL SEARCH REPORT

International application No.

PCT/IB 99/00495

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 25-28 are, as far as in vivo methods are concerned, directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. Claims Nos.: because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
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Remark on Protest

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/IB 99/00495

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9822504	A 28-05-1998	AU 4964097 A	10-06-1998